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## CRYSTALLINE ESTER CHOLESTEROL AND ATHEROSCLEROSIS

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BOSTON

THERE is a certain passive resistance among the medical profession to the consideration of evidence that arteriosclerosis is other than a wear and tear disease. This is understandable in the older group of medical men one of whom characterized the lesions of arteriosclerosis as "just rust." It is more difficult to account for in younger men engaged in progressive medicine. In the early period of medical history the thickening and calcification of the arterial walls in the aged was looked on as a natural phenomenon. The occurrence of such changes in younger persons was considered as due to more effective wear and tear. This obsession was not seriously disturbed when Virchow<sup>1</sup> in 1856 called attention to the form of arteriosclerosis with fatty lesions. It was not until 1904 that Marchand<sup>2</sup> gave this form a name "atherosclerosis." The studies of Aschoff<sup>3</sup> demonstrated that the fatty lesions contained crystals which were found to be those of crystalline ester cholesterol. This opened the door for nutritional studies by the Russian school. These studies began to be successful when atherosclerosis was produced in rabbits by feeding them egg yolk. Finally Anitschkow and Chalutow<sup>4</sup> produced the disease by feeding rabbits cholesterol in oil. This work was not accepted, largely because of the influence of Aschoff who had adopted Virchow's "imbibition" theory. The "imbibition" theory makes cholesterol an agent secondary to the wear and tear caused by the constant flow of blood on the arterial intima. Aschoff<sup>5</sup> was in doubt whether the relation of the crystalline esters to the lesions was causal or casual and his publications dampened interest in the cholesterol factor.

It is now generally accepted that crystalline ester cholesterol is constantly present in the active lesions of atherosclerosis.

From the Mallory Institute of Pathology

1, Virchow, R. Phlogose und Thrombose in Gefass-system in gesammelte Abhandlungen zur wissenschaftliche Medizin, Frankfurt, F. Meidlinger Sohn & Co., 1856

2 Marchand, F. Verhandl d Kong f inn Med **21** 23, 1904

3 Aschoff, L. Verhandl d deutsch path Gesellsch **10** 106, 1907

4 Anitschkow, N., and Chalutow, S. Centralbl f allg Path **24** 1, 1913

5 Aschoff, L. Lectures in Pathology, New York Paul B. Hoeber, 1924

In the intervening years evidence has been presented (a) that crystalline ester cholesterol is an irritant comparable to silica in provoking the growth of fibrous tissue<sup>6a</sup>, (b) that atherosclerosis is apparently prevented in the arteries of youth by the removal, before lesions are produced, of crystalline esters already deposited in the arterial intima<sup>6b</sup>, (c) that atherosclerosis appears in the cholesterol-fed rabbit after a long latent period during which excess esters in crystalline form are precipitated in liver cells in increasing amounts, are taken over by Kupffer cells that escape from the sinusoids when loaded, traverse the lungs and carry the esters into the subendothelial layer of the arterial intima<sup>6c</sup>, (d) that the lesions of the coronary arteries and of the myocardium of the rabbit fed cholesterol are particularly comparable to the lesions observed in human coronary arteriosclerosis<sup>7</sup>, (e) that the sequence of lesions and their relation to crystalline ester cholesterol can be followed in the human aorta from beginning invasion of cholesterophages to advanced fibrosis, scarring, necrosis and calcification<sup>6d</sup>, (f) that thyroid function controls cholesterol metabolism<sup>8</sup>, that if thyroid function is lowered by the use of thiouracil, atherosclerosis can be produced in the highly resistant dog by feeding cholesterol<sup>9</sup>.

Observations indicate that atherosclerosis is almost exclusively a human disease among mammals<sup>10</sup>. It is necessary to descend the scale to the birds, particularly to the domestic fowl (over 6 months old fed for market), to find the disease occurring naturally and commonly<sup>11</sup>. The disease is readily produced experimentally in young chickens by feeding cholesterol<sup>12</sup>.

At this point it is in order to indicate what is meant by the term "crystalline ester cholesterol". There occur in the cells of the normal adrenal cortex under polarized light brilliant granular points which are seen to be minute spheroidal crystals that show Maltese cross markings. If a frozen section of the organ is heated and gently compressed these crystals can be set free from the cells as fine droplets

6 Leary, T. (a) *Arch Path* **32** 507, 1941, (b) **37** 16, 1944, (c) **17** 453, 1934, (d) **21** 419, 1936

7 Wolkoff, K. *Beitr z path Anat u z allg path* **82** 555, 1929, **85** 386 1930. Leary<sup>6c</sup>

8 Murata, M., and Katooka, S. *Verhandl d jap path Gesellsch* **7** 27, 1917. Liebig, H. *Arch f exper Path u Pharmacol* **159** 265, 1930. Turner, K. B. *J Exper Med* **58** 115, 1933.

9 Steiner, A., and Kendall, F. *Arch Path* **42** 433, 1946.

10 Fox, H. in Cowdry, E. V. *Arteriosclerosis*, New York, The Macmillan Company, 1933. Zinserling, M. D. *Virchows Arch f path Anat* **213** 23, 1913. Zinserling, M. D., and Krimitzky, I. M. *ibid* **252** 177, 1924.

11 Dauber, D. V. *Arch Path* **38** 46, 1944.

12 Dauber, D. V. and Katz, L. N. *Arch Path* **34** 937, 1942.

of a semifluid material which tend to fuse together into larger crystals with the same markings. There is general agreement that these are cholesterol ester crystals. This is also true of the similar crystals found less constantly in the interstitial cells of normal testicles and the cells of corpora lutea. In the cholesterol-fed rabbit hypercholesteremia will be followed by the esterification and deposition of crystalline ester cholesterol in the animal's liver, from which it will be removed by scavenger cells and deposited in the arterial intima. Pressure on frozen sections of the intima harboring these cells will set free typical spheroidal crystals with the same markings, which fuse into larger crystals, just as happens with the cells of the adrenal cortex so treated. Moreover where the arterial deposits of these cells become too large to be supplied adequate nutrition and physical support, massive necrosis of cells will occur, the spheroidal crystals will be set free, the esters will be split, the fatty acids absorbed, and there will remain in the detritus of dead cells typical elongated rhomboid solid crystals of cholesterol. In atherosclerosis in man similar deposits of crystal-bearing macrophages will occur in atherocheumas, and mass necrosis will be followed by the deposit of typical solid cholesterol crystals in the debris of dead cells. These crystals are unlike crystals of other substances met with in the animal body.

From the chemical point of view the objection is raised that the spheroidal crystals which are present in the lesions of atherosclerosis may not be actually cholesterol ester crystals. What is the reason for this objection? According to Lison,<sup>13</sup> the attitude of the chemist has been negativistic with reference to the value of histochemical studies of the lipids. It has been found that cerebrosides and phosphatides may produce spheroidal crystals which cannot be differentiated morphologically from the crystals found in atherosclerosis or for that matter, from the crystals found in the adrenal cortex. Through the courtesy of Dr. S. J. Thannhauser and the Thannhauser Laboratory examples of cerebrosides, sphingomyelin and hydrolecithin were obtained. When heated with sodium oleate solution the cerebrosides produced spheroidal crystals in quantity, the sphingomyelin produced few crystals and the hydrolecithin rare crystals. How likely are these substances to interfere with conclusions concerning the reactions found in the cholesterol-fed rabbit or with those concerning the atherosclerotic findings in man? Cerebrosides arise largely in nerve tissue, tend to be fixed in cells and are mobilized in quantity (kerasin) only in the rare Gaucher's disease. Similarly, sphingomyelin is likely to be mobilized in quantity only in rare congenital familial Niemann-Pick disease. Moreover, the cerebrosides and sphingomyelin or lecithin require union with fatty acids before spherocrystals are produced. It is recognized that, apart from

neutral fats, almost all the intracellular fatty acids are present in cholesterol esters. By no possibility could esters of other lipids leave in the tissues a residue of solid cholesterol crystals when the esters are split. At best they might be mixed with true cholesterol ester crystals as diluents, but they could not affect the fundamental specificity of the crystalline ester cholesterol in its relation to the lesions of atherosclerosis in man or in the cholesterol-fed rabbit. It is a reasonable deduction that the substance which closely duplicates the spheroidal crystals of the adrenal cortex (accepted as crystalline ester cholesterol) and which yields characteristic solid crystals of cholesterol when the cells carrying it break down is entitled to be referred to as crystalline ester cholesterol. The crystals are called crystalline cholesterol esters to distinguish them from esters in the blood which have less birefringence, if any.

In my experience, studies of material in a service dealing with deaths by violence and sudden deaths disclosed that, with due allowance for syphilitic occlusion of coronary orifices for rheumatic or infectious arteritis, for thromboangitis obliterans and for periarteritis nodosa, atherosclerosis is responsible for at least 90 per cent of coronary arterial disease. The clinical approach to coronary disease was based until recently on the belief that all arteriosclerosis was a wear and tear disease, inevitable and incurable. All efforts were expended on the improvement of methods of diagnosis. With the advent of the electrocardiogram, questions of the etiologic factors of the disease were given a little attention. Recently, as information increased, the possibilities became probabilities that atherosclerosis is a specific disease. The present situation demands demonstration, beyond reasonable doubt, that cholesterol is, or is not its cause. It is almost unnecessary to say that any hope of removing atherosclerosis from the group of inevitable incurable diseases must arise from studies of the cause and the method of action of that cause. Fortunately, from this point of view atherosclerosis is a chronic, intermittently progressive disease. It is possible to put together in orderly sequence, what Sydenham referred to clinically as the footsteps of disease. Studies of atherosclerosis particularly of coronary lesions supply data as to how the causative agent produces its effects and permit recognition of the relation of the terminal phases of the disease to that agent after it has disappeared from the lesions.

Because of the intricate character of the cholesterol molecule which Schoenheimer (to whom present investigators owe much for basic studies on cholesterol) classified as one of the most complex substances occurring in the animal body, chemistry has had little to offer in the solving of the atherosclerotic problem. The use of isotopes may be helpful but the striking characteristics of crystalline ester cholesterol permit it to be identified and followed in its transport until it is deposited in new sites in the tissues without the use of isotopes.

It is desirable at this time to review with more complete evidence and with wider knowledge of the characteristics of crystalline ester cholesterol the etiologic relation of this substance to human and experimental atherosclerosis.

Cholesterol is distributed universally in the cells of the human and the animal body and is particularly abundant in brain and cord (myelin) and fat tissue. It is probably in organic combination in the cells, for the most part and is not visible under polarized light with ordinary equipment, because of its low birefringence, if any. Crystalline esters and solid crystals on the contrary are strongly birefringent (fig 1 *A*). In the normal human body crystalline esters are found in the adrenal cortex and (not constant) in the interstitial cells of the testicle and the cells of corpora lutea.

Under pathologic conditions, crystalline ester cholesterol is precipitated focally when tissues rich in cholesterol notably brain and cord and fat tissue undergo necrosis. It occurs within tubular epithelium in the kidney in glomerulonephritis in late fatal lipoid nephrosis in certain adult renal tumors and in other new growths under various conditions. It is found in macrophages in Hand-Schüller-Christian disease and the related Letterer-Siwe disease. Excessive cholesterol intake or inadequate cholesterol metabolism or both may give rise to excessive deposits in macrophages of crystalline ester cholesterol in the subendothelial layer of the arterial intima or to more diffuse deposits, as in xanthomas.

In my experience, studies of human tissues and of tissues of experimental animals have demonstrated that in frozen sections of fresh tissue or of tissue recently fixed in formaldehyde solution there will ordinarily appear only two forms of strongly anisotropic crystals, i. e., crystalline ester cholesterol and fat crystals. Solid cholesterol crystals may be found in advanced lesions of arteries and in xanthomas. The term "fat crystals" is used without reference to the chemistry of the crystals, concerning which, according to Lison,<sup>13</sup> there is considerable doubt. The commonest examples occur within fat drops in fatty cells. They are made up of needle crystals frequently bundled into sheaves (fig 1 *B*). Moderate heating of the slide causes these crystals to disappear, to reappear as the slide cools. On the other hand crystalline ester cholesterol is brought out more clearly by heating. Too high temperature or too long exposure will affect even crystalline ester cholesterol. Sometimes cholesterol crystals appear in needle forms, particularly after tissues have been fixed in formaldehyde solution or frozen for section work. Heating may reconvert the rod forms into typical spheroidal crystals. In tissue kept in acid formaldehyde solution or for long periods in neutral formaldehyde solution a multiplicity of varied crystals may arise with no typical crystals, and reversion



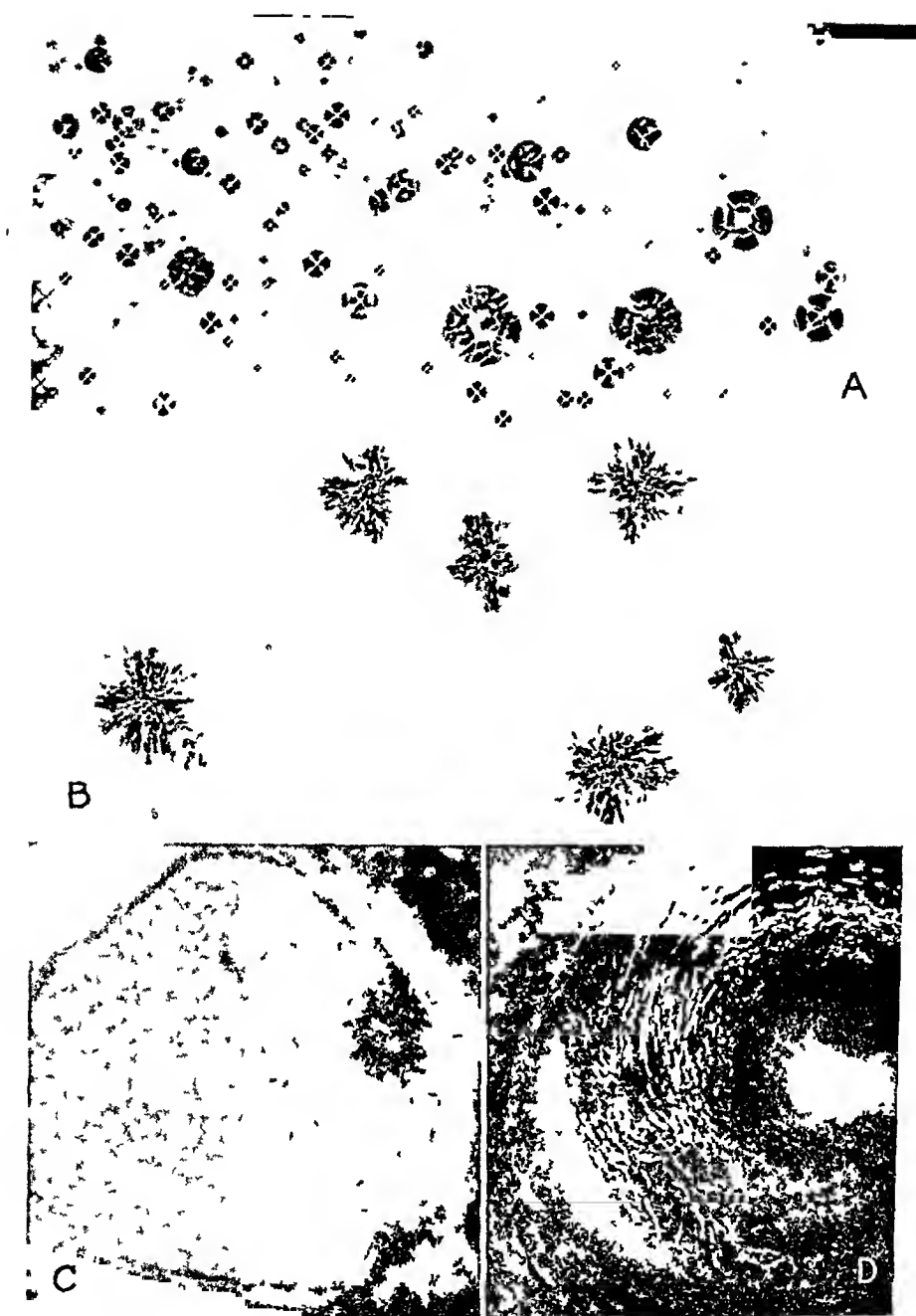


Fig 1—*A*, cholesterol ester crystals extruded from cholesterophages into the lumen of an advanced human coronary lesion with a small active focus. Unstained frozen section,  $\times 350$ . The large masses are globules compressed between slide and cover slip that have lost symmetry.

*B*, fat crystals in drops of fat in fat cells. Unstained frozen section of human epicardial fat,  $\times 350$ .

*C*, Kupffer cell lying in a sinusoid of rabbit's liver. Paraffin section of tissue fixed in Zenker's solution, stained with Mallory's phosphotungstic acid-hematoxylin,  $\times 800$ .

*D*, solid cholesterol crystals in fibrotic intima of a coronary artery (see text). Unstained frozen section under polarized light,  $\times 30$ .

by heat is impossible. It follows that for the study of crystalline ester cholesterol and its relation to disease it is necessary to use sections of fresh tissue or tissue fixed in neutral formaldehyde solution for short periods.

Crystalline ester cholesterol provokes phagocytosis. The crystals are rarely found as free bodies. They are engulfed by macrophages—Kupffer cells if they are precipitated in the liver and histiocytes if they are set free in other tissues. In both types of cells the esters are divided into minute globules, 1 to 3 microns in diameter, as they are engulfed and are then distributed evenly throughout the cytoplasm of the macrophage. The cells have a finely granular appearance under polarized light.

In the embedding of tissues in paraffin or celloidin (a concentrated pyroxylin), the defatting process extracts crystalline ester cholesterol with other lipids, and the cholesterophages become typical foam cells (fig. 1 C). Solid crystals, as stated, occur in tissues in which cholesterophages have undergone necrosis en masse. They represent end products, not readily dissolved, tending to persist in tissues for long periods. As an irritant they provoke a fibroblastic reaction, sometimes with formation of giant cells.

Figure 1 D shows a lesion of a coronary artery of a man, M. P., 45 years of age who collapsed in the shipping room at his place of employment. He was removed to a hospital and on arrival was pronounced dead. He was 5 feet 7 inches (170 cm) tall and weighed 180 pounds (81.5 Kg). He had not seen a doctor but had complained of indigestion and was seen buying "tums" (a preparation containing calcium carbonate, magnesium carbonate, magnesium trisilicate, oil of peppermint and sugar) in a drugstore at noon on the day he died (at 4 p.m.). Autopsy disclosed a heart weighing 400 Gm with distended cavities filled with "currant jelly" clot and fluid blood. The left coronary artery and anterior descending branch showed thickening of the wall almost to occlusion 3 cm below the orifice. In the circumflex branch and the right coronary artery there was thickening of the wall, but the lumen was free and of moderate size. In the myocardium there was no gross evidence of fibrosis, and the microscope revealed only focal lymphoid cell infiltration about some vessels. Other organs were not remarkable except the liver, which weighed 2,860 Gm and was rich in fat, with early periportal fibrosis and lymphoid cell infiltration. The coronary lesion was marked by fibrosis with broad collagen bands beset with typical solid crystals of cholesterol. The picture suggests repair of an atherocheuma. Death was due to coronary insufficiency.

As described in an earlier publication,<sup>6a</sup> the long latent period, after the beginning of cholesterol feeding in the rabbit, before visible arterial lesions appear has permitted detailed studies of the early progressive stages of the experimental disease. Certain phases of this development need to be emphasized. Cholesterol is the only known agent which will cause atherosclerosis when fed to rabbits, chickens or dogs. (For discussion of other substances, introduced intravenously, see "Comment," page 25.) Cholesterol or animal cholesterol freely passes the intestinal

barrier. Most vegetable sterols cannot pass this barrier (Schoenheimer), with ergosterol the exception to the rule. The source of dietary cholesterol is the animal fats, including milk fats, egg yolk, lard and brain and cord. In liver cells the esterification of cholesterol is a normal function, recognized by Thannhauser and Schaber<sup>14</sup> in 1926. The depositing of visible esters in liver cells as in the rabbit, is due apparently to cholesterol in excess, and is the first lesion to appear (after hypercholesteremia) in that animal. Figure 2 *A* illustrates the distribution of fat-staining material in the rabbit's liver. The process begins in the cells at the centers of lobules and spreads toward the peripheries of the lobules as the material accumulates. In figure 2 *B*, this fat-staining material is seen to be crystalline ester cholesterol by polarized light, in a relatively early stage of deposition.

The functioning of the Kupffer cells in the removal of the excess crystalline ester cholesterol is remarkable. The basic function of these cells is the removing of particulate foreign bodies from the circulating blood. Experimental studies of the process of foreign body removal have been carried out with many varieties of particulate matter which was injected intravenously in suspension. The work of Beard and Rous<sup>15</sup> is most informing. It disproved the belief that Kupffer cells were attached to the sinusoid wall by tenuous, easily broken connections. Efforts to wash these cells out of the sinusoids of the normal rabbit's liver in quantity were not successful. On the other hand, washings were rich in cells when the cells had taken up foreign bodies. Studies disclosed that the cells were large, with an immense capsule-like circular membrane that might reach a diameter of 100 microns, that the cells were very sticky, tending to adhere to one another or to other cells and causing anything with which they came into contact to adhere to their surfaces, that they thrived best in culture when lens paper, to which they could cling, was introduced into the culture fluid.<sup>16</sup> In a word the cells were large, were capable of great increase in size, were sticky and tended to cling to other structures.

In experimental atherosclerosis there are no demonstrable foreign bodies in the blood stream. The removing of ester crystals from the liver cells is a reversal of standard practice. In figure 2 *C*, illustrating a quite advanced hepatic deposit of crystalline ester cholesterol, the cleancut character of the vacuoles that had contained crystalline ester cholesterol is manifest. These vacuoles may be 10 or more microns in diameter in contrast to the minute vacuoles that occupy the Kupffer cells. The engulfing of crystalline ester cholesterol piecemeal in minute droplets from the large drops in the liver cells is probably facilitated by the semifluid consistency of the material.

14 Thannhauser, S. J., and Schaber, H. *Klin. Wchnschr.* 5: 252, 1926.

15 Beard, J. W., and Rous, P. *J. Exper. Med.* 59: 593, 1931.

16 Rous, P., and Beard, J. W. *J. Exper. Med.* 59: 577, 1931.

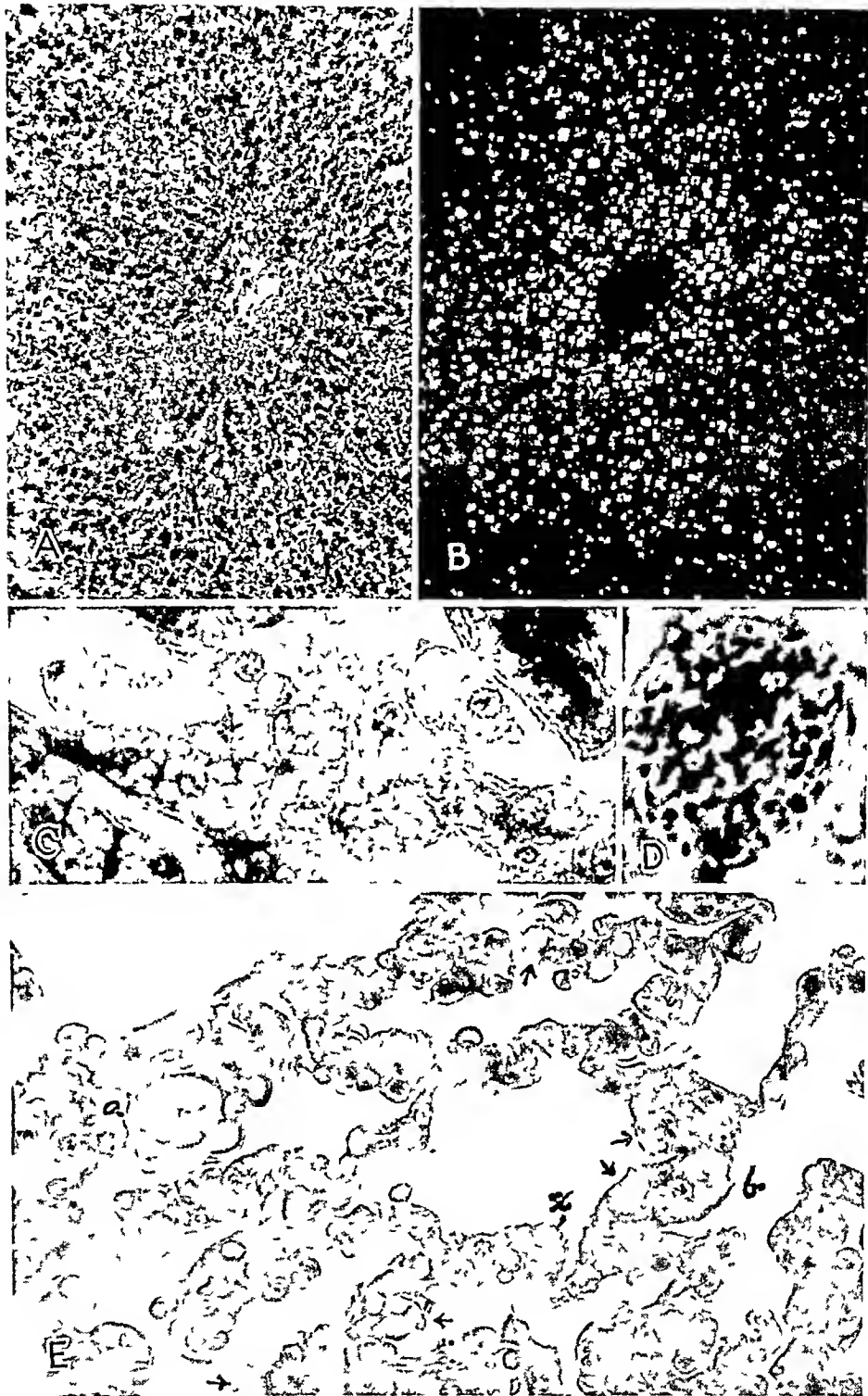


Fig 2—*A*, frozen section of a liver lobule of a rabbit stained with sudan IV,  $\times 90$

*B*, frozen section of a liver lobule of a rabbit, unstained, under polarized light,  $\times 80$

*C*, liver of rabbit fed cholesterol—advanced lesion. Vacuoles that contained crystalline ester cholesterol are sharply outlined. Compare the size of these vacuoles with the fine droplets in the cholestero-phages in the dilated sinusoids. Zenker solution fixation, paraffin section, Mallory's phosphotungstic acid-hematoxylin stain,  $\times 350$

*D*, cholestero-phage in a pulmonary artery. Frozen section stained with sudan IV and hematoxylin,  $\times 600$

*E*, cholestero-phages in transit through lung capillaries. Note two resting cells, *a* and *b*, a cell, *c*, following a kink in a capillary, and portions of other cells coming into the plane of section. Zenker solution fixation, paraffin section, Mallory's phosphotungstic acid-hematoxylin stain,  $\times 350$

The scavenger function of the Kupffer cells requires that the crystalline ester cholesterol be removed from liver cells to new sites of deposit. The avenues by which the scavenger cells may escape from the sinusoids are the blood and lymph channels. In the early stages the cells are delivered into the circulation almost exclusively through the blood vessels, as would be expected. In figure 2 *D* such a cell with fat-stained contents is seen lying free in the pulmonary artery of a rabbit. If the sinusoids become plugged with swollen Kupffer cells, these will escape through the liver tissue to the lymphatic channels in the periportal connective tissue. Indeed, in the clearing of liver cells of crystalline ester cholesterol after cholesterol feeding has been stopped, lymphatic channels may arise about the central veins in some cases.

The passing of Kupffer cells through the capillary system of the lungs needs little comment. The wide, poorly supported capillaries offer open conduits to cells whose amoeboid activities can carry them through the interstices of solid tissue. Rarely obstruction may arise behind occluding cells and a capillary infarct be produced. Figure 2 *E* illustrates the lung transit. Two resting cholesterophages (*a* and *b*) have withdrawn their processes and have ballooned out capillaries or small veins. Elsewhere a phage has elongated itself and is following a kink of a twisting capillary (*x*). Portions of other phages (arrows) lie in the plane of the section.

It would seem that the loaded Kupffer cell has but to release itself from the sinusoidal wall to enter freely a hepatic vein. The variation in the number of cholesterophages found in the lungs of rabbits during the period when these cells should be passing through these organs throws doubt on this thesis. During most active passage it was apparent that the transit was made by the cells in showers. Figure 3 *A* shows massed cholesterophages at the junction of pulmonary veins. Between showers there were found few cells, apparently left over in backwaters from previous mass passages. A central mechanism which relaxes sinusoidal walls would best account for the conditions observed.

As the Kupffer cells pass through the lungs, there is a shrinkage in the size of the cells with loss of crystalline ester cholesterol (fig 3 *A*). After the invasion of the subendothelial layer of the intima the cell content of crystalline ester cholesterol is reestablished in a manner which is not clear.

The freed Kupffer cells find themselves in a closed system of vessels, pulmonary or systemic. Whether they are delivered from this system is dependent on the efforts of the cells themselves. Some escape from the capillaries of the lung into the alveoli in passage, but the number is small. Practically all the escaping cells appear to make their way out of the blood stream in the thick-walled portions of the arterial system. Their primary evasion, however, is only subendothelial, and they tend to

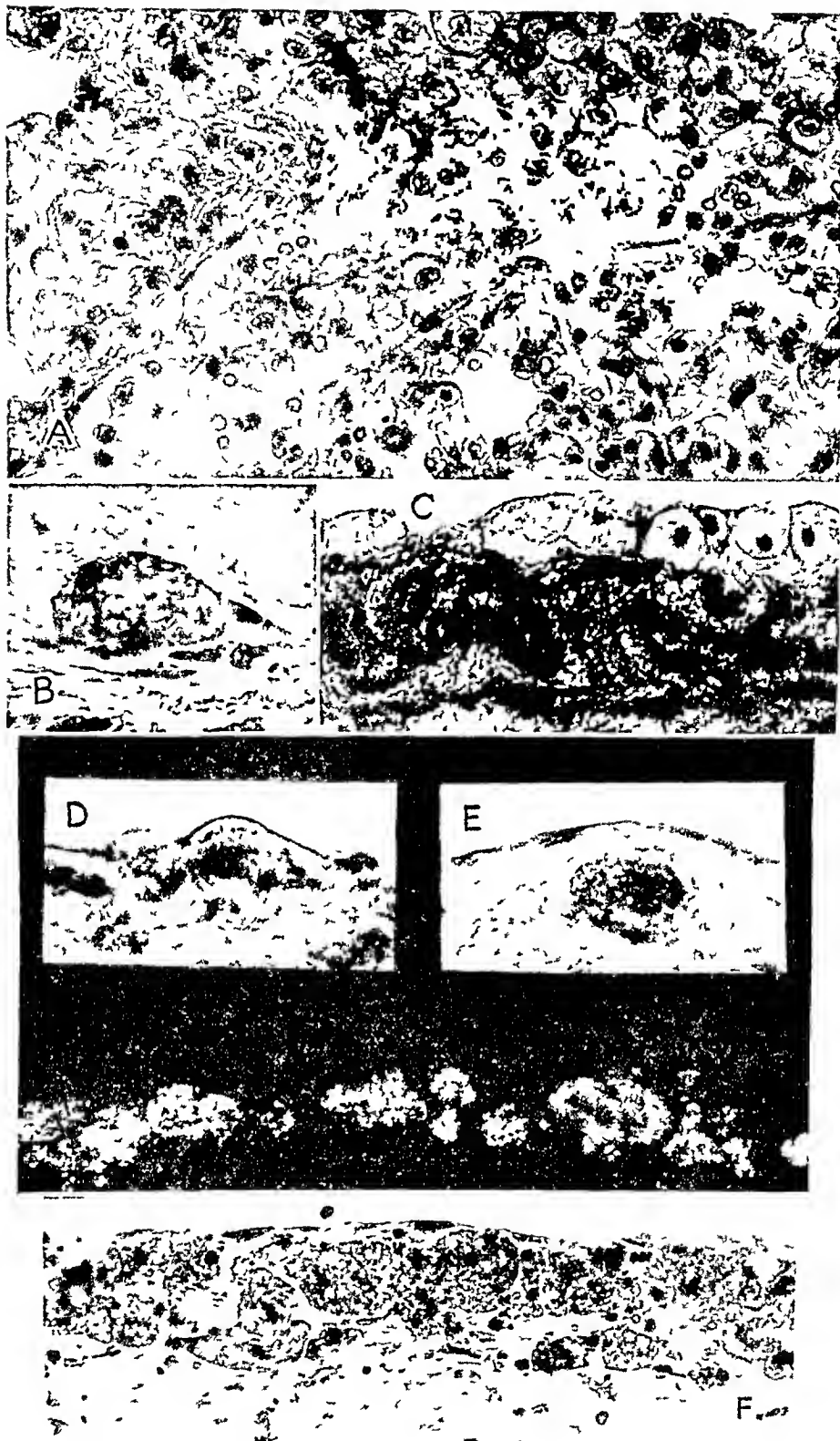


Fig 3—*A*, junction point of veins in a lung of a rabbit containing cholesterophages Zenker solution fixation, Mallory's phosphotungstic acid-hematoxylin stain,  $\times 350$

*B*, cholesterophage clinging to the wall of a rabbit's pulmonary artery and lifting the edge of an endothelial cell (see text) Frozen section, sudan IV and hematoxylin stain,  $\times 500$

*C* row of cholesterophages under the endothelium of a rabbit aorta Paraffin section, Zenker solution fixation, stained with Mallory's phosphotungstic acid-hematoxylin  $\times 120$

*D* and *E*, solitary cholesterophages from a human aorta *D* shows an early stage with a hump, *E*, a later stage, more deeply located Frozen sections, sudan IV and hematoxylin stain,  $\times 300$

Below *D* and *E* is a row of cholesterophages in the subendothelial layer of a human aorta as seen under polarized light,  $\times 200$

*F*, cholesterophages lying under the endothelium of a human aorta Frozen section, hematoxylin and sudan IV stain,  $\times 400$

remain within the intima as this increases in thickness in atherosclerosis. Occasional invasion of the media is met with in the coronary artery and is more common in advanced aortic lesions, notably atheroaneurysms.

The regions in which early lesions are most favored in the experimental rabbit and man are the ascending aorta, the epicardial portions of the coronary arteries, the regions surrounding the orifices of vascular branches, and the points of branching. Lesions of the ascending aorta are very common in man but are usually temporary. They occur as pin-head processes that may spread superficially but rarely progress to advanced lesions, since the crystalline ester cholesterol is removed from the macrophages by fibroblasts before permanent lesions result.

Gordon<sup>17</sup> has called attention to the possibility that the pulsatile intermissions of the arterial blood stream are related to the localization of atherosclerotic lesions. Where the flow is continuous, the blood stream is made up of an outer peripheral zone containing plasma without cells and a central axial zone containing cells in concentration. Cohnheim demonstrated the vascular changes in inflammation by exposing the mesentery of the frog to air under the microscope and more focally by point cauterization of the frog's tongue. Congestion with dilatation of vessels and slowing of the current abolished the zones in the blood stream, brought the leukocytes into touch with the vascular endothelium and thus enabled them to escape from the blood vessels. In the focal processes in the tongue it was evident that damaged tissue or the products of such damage were responsible for the call, the chemotaxis, that brought the leukocytes out of the vessels and through the tissues to the region of injury. However, the escape of cells was limited largely to the venules, veins and capillaries. Paradoxically the escape of Kupffer cells occurs in thick-walled arteries. Though chemotaxis has been suggested as the agency responsible for their escape, it does not explain what actually occurs.

The sites at which cholesterol-loaded cells accumulate in the arterial intima are in regions where the continuity of the zonal type of flow is interrupted. In the ascending aorta with the closure of aortic cusps there is a momentary, practically complete stoppage of the flow. In the epicardial portions of the coronary arteries the flow is slowed in systole because the contracting ventricles, particularly the left, are compressing the muscular branches which are thus prevented from receiving blood. Meantime the epicardial branches are distended with blood under systolic pressure. The resulting slowing of the blood should be great enough to overcome zonal flow. At the orifices of branches swirling currents arise which interrupt the zoning and at branching points splitting of the stream must interfere with the zonal flow. In short, the

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<sup>17</sup> Gordon, I. Arch Path 49 247, 1947

sites favoring early atherosclerotic lesions are regions in which interference with or interruption of the zonal flow permits the cholesterophages to make contact with the intimal endothelium

Contact with the endothelial surface having been made, there come into play the stickiness, the clinging qualities and the ameboid powers of the Kupffer cells, which cling to the wall under difficulties. The passing blood tends to wash them off the wall. As it streams by, it flattens and elongates the cells. Relief can come only by their escaping from the current. Figure 3 *B* illustrates an early stage in the invasion of the intima of the pulmonary artery of a cholesterol-fed rabbit. A Kupffer cell is clinging to the arterial wall. Its body is being compressed and stretched by the circulating stream. The cell appears to be prying up the edge of an endothelial cell, whose nucleus is seen to the right. The nucleus of the cholesterophage is along its left border. The cell contents are deeply colored by the fat stain. The lumen of the artery contains largely platelets.

Gordon<sup>17</sup> has expressed the opinion that the pressure of the blood pushes the cells as passive agents through the endothelial layer. To one who has studied loaded cholesterophages passing through the dense human or rabbit derma in xanthoma the claim that other powers than those possessed by the cholesterophage are needed to propel it into the subendothelial layer seems unfounded. A glance at the escaping cell of figure 11 will make evident that the cholesterophage is making its way, in spite of the activities of the flowing blood, toward the subendothelial layer of the intima.

It is a reasonable conclusion that the sites at which the earliest atherosclerotic lesions arise in the arterial system are regions where opportunity is first presented for cholesterophages to come into intimate contact with the arterial intima. Taking advantage of that contact the Kupffer cell with its sticky surface, clinging habit and ameboid powers makes its way from its hazardous position on the wall into the shelter of the subendothelial layer.

It is probable that the hump produced by such a cell as it lies directly under the endothelium is a projection that may furnish an alighting or anchoring place for other cells. At any rate, rows of cells and cell mounds are common in the subendothelial layer in man and in the rabbit fed cholesterol. An early row of such cells is illustrated in figure 3 *C* from a rabbit aorta.

Thus far I have dealt with the early stages of atherosclerosis as produced in the rabbit and the method of its production. Advanced stages duplicate human lesions and are illustrated elsewhere. No demonstration of the process by which human liver cells produce excess esters and precipitate the crystalline esters in liver cells, together with the activities by which Kupffer cells remove and transport these esters,



has been made. The long latent period, the intermittent character of the human lesions, the unlikelihood that any human being would adopt a diet as continuously rich in cholesterol as that fed to the rabbit, and the relatively enormous human liver, compared with that of the rabbit, are probably responsible for this failure. However, the early lesions of human atherosclerosis correspond so closely in location, character and development to the early lesions in the experimental rabbit that it is a reasonable deduction that they are similarly produced. The cholesterophage invasion of the subendothelial layer of the intima, the formation of rows and solid nodules of cells with little fibroblastic support, the progressive fibrosis that strangles the cholesterophages as it increases and the formation of typical nodules that go on to scarring and calcification have been illustrated elsewhere as well as in this publication (See following pages.)

#### HUMAN LESIONS

Discussion of human atherosclerotic lesions is of limited value without the demonstration of actual specimens or reproductions. The material in the following pages illustrates how the atherosclerotic lesions are related to crystalline ester cholesterol, the cumulative character of the disease in man, because of intermittent overdosage of cholesterol, and some of the mutations occurring in processes at various ages.

*Aortic Lesions*—The illustrations of lesions of the human aorta here presented deal only with early processes since representative lesions covering almost the whole gamut of the progressive stages of the disease from early deposits through advanced stages was presented in an earlier publication<sup>6d</sup>. Malloy has said "In order to understand a pathological process it is necessary to study its beginning and to trace its biologic development." In the present publication the illustrations reproduce literally the beginnings and the early biologic development of aortic sclerosis.

The beginnings are best studied in the regions where early pin-head lesions are present in crops, as in the ascending or other parts of the aorta. Besides the lesions that are grossly visible, series of frozen sections will disclose under the microscope lesions that are not visible to the naked eye. The earliest finding deals with the solitary cholesterophage which is making its way into the subendothelial area or has arrived in that layer. In figure 3, parts *D* and *E* illustrate two such cells. *D* shows a cell which has succeeded in getting under a mantle made up evidently of the thin body of an endothelial cell, it still presents a hump above the level of the endothelial surface. It is unfortunate that the red color of the sudan IV stain cannot be reproduced since it adds authority to the cell. *E* shows a similarly isolated cell that has come to rest in the subendothelial layer of the intima. Both of these cells were found in

the ascending aorta of a woman of 42 years. They occurred among small and large (pinhead) nodules scattered loosely over a large surface of the aortic intima.

Below *D* and *E*, in figure 3, there is reproduced a row of cholesterophages lying in the subendothelial layer as seen in a section of a small flat nodule in the ascending aorta. This lesion was flat because it was made up largely of a single layer of cells. The knife has passed through these cells, which were for the most part large, but has cut not only median sections through the cytoplasm but also sections through outer portions of cells, and this accounts for the variation in size. The finely granular appearance of the cells is due to their content of minute dioplets of crystalline ester cholesterol. In a later stage the pinhead nodules tend to form flattened mounds with the thickest layer of cholesterophages in the midregions.

In a stained section of a later lesion (fig. 3 *F*) the relative positions of cholesterophages and the endothelial layer of the intima are illustrated. These cholesterophages, larger than the massed cells in the pulmonary veins (fig. 3 *A*), illustrate also their tendency to increase their crystalline ester cholesterol content after coming to rest in the subendothelial layer. This particular lesion is not a primary lesion. In the deep layers are fragments of cells which have taken the sudan IV stain. They appear to be either the remnants of cholesterophages which had invaded the subendothelial layer at an earlier moment and had undergone disorganization or the remains of fibroblasts which had taken over crystalline ester cholesterol from the disorganizing cholesterophages.<sup>6a</sup> The row of cells represents, then, a reactivation of an older lesion. Figure 4 *A* is from a section of the midregion of a pinhead lesion. There are now several layers of cells. The section technic has led to pressure being exerted on the cells, particularly in the lower layers. Rupture of the cytoplasm in many cells has set free ester cholesterol crystals that have fused into larger crystals, leaving no doubt concerning the character of the cell contents. Many crystals still are held within the capsules of cells.

The sequence of parts *B*, *C*, *D* and *E* of figure 4 has to do with the appearance and relation of the cells of pinhead lesions, together with the character of the cell contents. In *B* and *C* are shown sections of a pinhead lesion. The upper section has been stained with sudan IV and hematoxylin. The deep staining cholesterophages tend to occur in rows as they frequently align themselves in such lesions. The thickening of the intima to accommodate the cells has been accompanied by a minor degree of fibrosis, adequate to support the cholesterophages and no more. The lower section is a neighboring one and is seen unstained under polarized light.

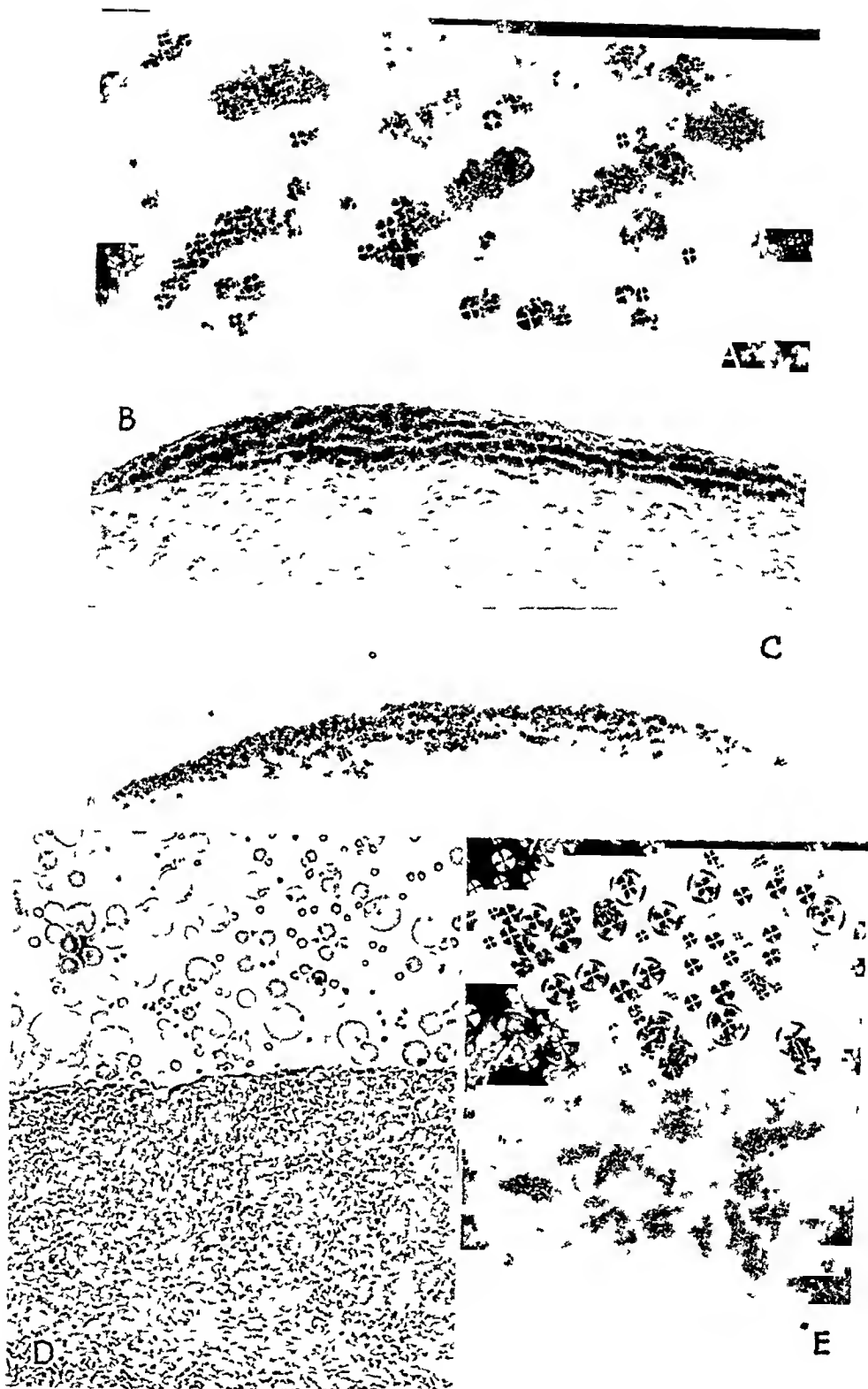


Fig 4—*A*, rows of cholesterophages in the intima of a human aorta. Frozen section, unstained,  $\times 150$

*B* and *C*, frozen sections of pinhead lesions of a human aorta. The upper section is stained with hematoxylin and sudan IV, the lower, unstained, is seen under polarized light,  $\times 30$

*D*, frozen section of a pinhead lesion of a human aorta, warmed and compressed to free drops of greaselike material, unstained, under diffuse light,  $\times 200$

*E*, same field under polarized light

In *D* and *E* the results of heating the slide and delicate pressure are manifest. In *D* the slide, unstained, under diffuse light makes clear the greaselike material, softened under heating, insoluble in glycerin or glycerin jelly or water, that can be expressed from the cells. The variation of size of the drops and dioplets is dependent on fusing of some dioplets into larger drops. In *E* (the same field under polarized light) the finely granular appearance of the cholesterophages in the intima still persists for the most part. They have not been completely robbed of their crystalline ester cholesterol contents. The finest dioplets, as they naturally occurred in the cytoplasm of the cholesterophages, can now be seen in their polarized images, though they cannot be made out in the section photographed under diffuse light. They represent a diameter of 1 to 3 microns of significance since Gardner and Cummings<sup>18</sup> demonstrated that the efficient crystals of silica producing silicosis were 1 to 3 microns in diameter.

*Lesions of the Coronary Arteries*—In rabbits fed cholesterol the daily excess of cholesterol may lead to the production of massive acute lesions in which many layers of cholesterophages occur with the cells enveloped in a delicate fibroblastic meshwork. These acute lesions are rare in man. However, they were found in case 1.

CASE 1—S. S., a doctor of veterinary medicine 25 years of age married, had suffered from indigestion for years and had been put on an ulcer diet rich in milk and cream, though roentgenographic evidence was negative for ulcer at Bellevue Hospital. He had complained of indigestion two months before death and was treated by a local physician. He was talking with his wife in his veterinary hospital when he said he had an acute pain in the abdomen and fell on the floor. A rescue squad of the Boston fire department used a respirator without avail. His local physician pronounced him dead within ten minutes. Autopsy disclosed obliterative pericarditis. Though the adhesions were firm, the pericardium was thin. The heart and pericardium weighed only 380 Gm. The valves and the cavities were normal. There was no myocardial fibrosis, nor were any Aschoff nodules found.

There was thickening of the walls of the left coronary artery, with yellow deposits in the main vessel and the branches. There was considerable narrowing of the lumen of the descending branch of the left coronary artery, but there was no thrombosis. The right coronary artery showed minor yellow deposits.

Microscopically, the coronary lesions were unusual in that massive collections of cholesterophages with threadlike bands separating the cells were found in some lesions (fig 5 *A*) and multiple lesions of different ages were present in the same section (fig 5 *B*). Such findings are characteristic of the more acute forms of atherosclerosis occurring in some rabbits fed cholesterol.

There was no evidence of active or healed peptic ulcer.

The earlier lesion illustrates the delicate fibroblastic meshwork in which the cholesterophages lie. In the more advanced process (fig 5 *B*) the thickening of the fibroblastic meshwork has strangled many of the phages which have disappeared. Remnants of these cells and a few larger cholesterophages are seen in

18 Gardner, L. V., and Cummings, D. E. *Am J Path* 9:751, 1933.

the otherwise dense fibrous meshwork. The older part of the lesion, to the right, nearest the media, has undergone scarring and necrosis of the dense fibrotic tissue. Facing this advanced lesion, on the opposite wall, is a second nodule of about the same age. These older lesions, as they thickened the wall, came almost into contact, narrowing the lumen. In the immediate subendothelial space over this second nodule a new group of cholesterophages is producing a reactivation of the lesion.

It is possible to follow in these very active lesions the progression of the lesion from the initial invasion of cholesterophages which come to lie in a delicate fibroblastic meshwork. The meshwork becomes more dense as the crystalline ester cholesterol



Fig 5—*A*, early human coronary lesion showing cholesterophages with a delicate fibrous network. Zenker solution fixation, paraffin section, Mallory's phosphotungstic acid-hematoxylin stain,  $\times 350$ .

*B*, moderately advanced human coronary lesions (at right) with a fresh invasion of cholesterophages beneath the endothelium reactivating the advanced lesion to the left. Same technic as for the lesion shown in *A*.

contents of the cells stimulates fibrosis at the expense of the cholesterophages, which shrink and finally disappear under pressure, leaving a scarred tissue with few cholesterophages remaining. The end result may be fibrosis without evidence of surviving cholesterophages or crystalline ester cholesterol.

In this case the symptoms of indigestion were accepted as evidence of peptic ulcer in spite of negative roentgenologic findings. Indigestion may be the first

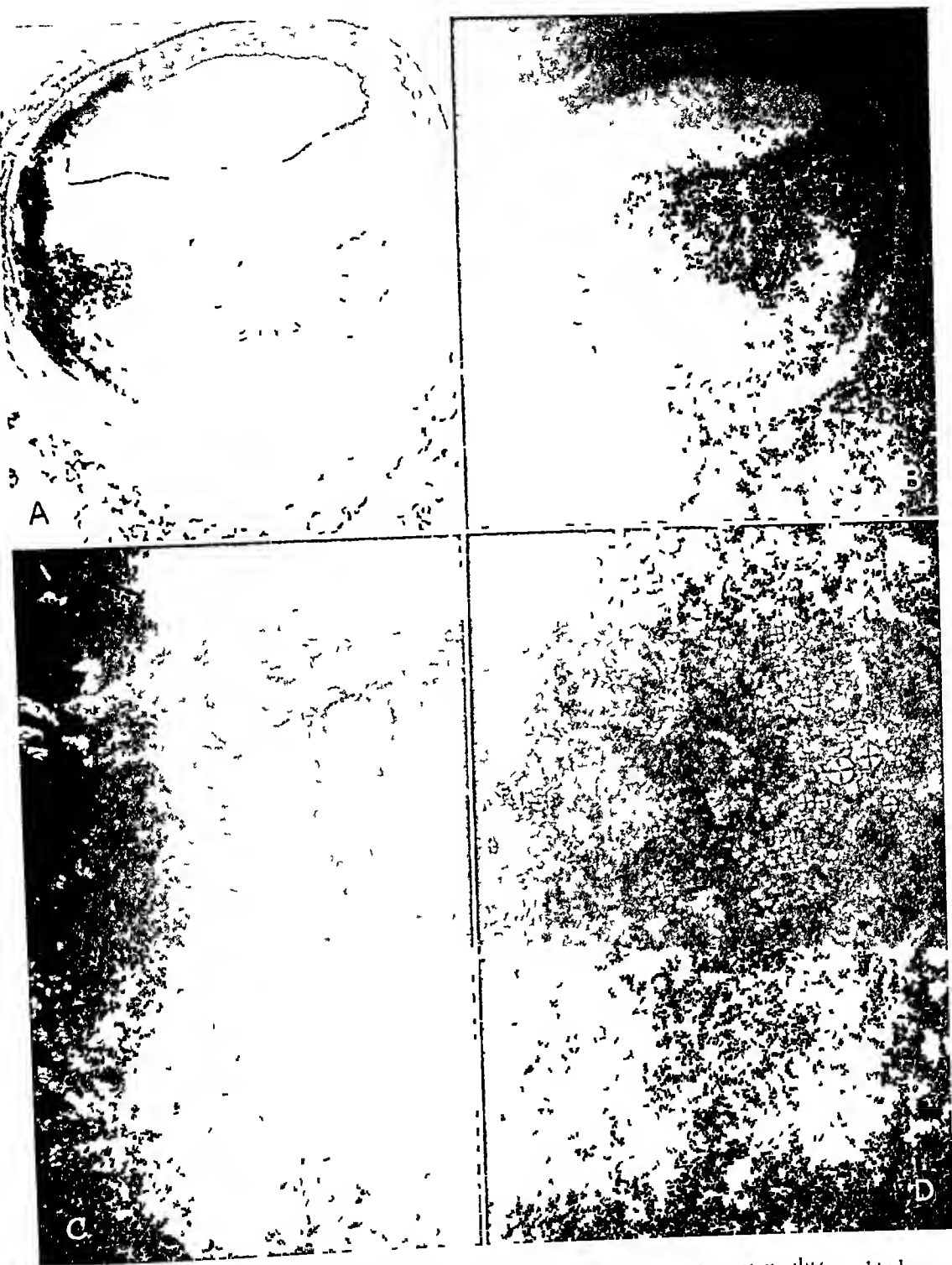


Fig 6—*A*, frozen section of a human coronary artery with early atherosclerosis near the lumen and fat-staining material in two layers below. Hematoxylin and sudan IV stain,  $\times 60$

*B*, same lesion under polarized light,  $\times 60$

*C*, enlargement of the two layers of doubly refractive material shown in *B*, under polarized light. Cholesterophages below, solid crystals above.  $\times 250$

*D*, crushed area of a cell layer seen in *C*, under polarized light. Some uncrushed cells are seen below to left. Crystalline esters, small and larger (fused) crystals, notably to the right and above.  $\times 300$

and sometimes the only symptom of coronary disease up to a critical, perhaps terminal point. The patient was treated intermittently with a Sippy diet, which could account for the unusual activity of the process.

CASE 2—S G, a captain of the fire department, 47 years of age, was crushed by a falling roof at a fire. The left coronary artery showed gross thickening of the wall and eccentric narrowing of the lumen. Microscopically (fig 6 A), the eccentric thickening of the intima is seen to be due to a cellular, fibrotic inner layer and an outer portion containing abundant fat-staining material, which occurs roughly in two layers. Under polarized light (fig 6 B) the two layers show distinct patterns. The outer layer near the media is made up of larger crystals which under higher magnification (fig 6 C) consist of solid crystals of cholesterol. (The greater thickness of frozen sections causes the crystals, which lie at various angles, to present broader surfaces than are seen in the slitlike spaces they occupy in thin paraffin sections.) The inner layer is made up of cholesterophages which invaded through the vascular endothelium and probably stimulated the formation of the cellular inner fibrous layer as they traversed the tissues toward the depths. That they are cholesterophages is seen in figure 6 D showing a frozen section of the lesion, unstained, which was warmed and then pressed, rupturing the cholesterophages and freeing the ester cholesterol crystals. In the deep layers about and internal to the solid crystal layer are collections of cells, largely elongated bipolar cells which contain fat-staining material that is not doubly refractive. These appear to be fibroblasts that may have been active in removing and dissolving crystalline ester cholesterol from cholesterophages of the earlier invasion.

This coronary artery illustrates the cumulative character of lesions with reactivation after long intervals.

CASE 3—L D R, an obese man, measuring 66 inches (167.5 cm) in length and weighing 250 pounds (113 Kg), was found dead. Death was due to hypertensive heart disease and coronary sclerosis. The heart weighed 520 Gm. The mitral valve was thickened along the contact edge, with embossed vessels over the anterior flap and some shortening and thickening of the chordae tendineae. The deformity of the valve was not marked. The left coronary artery was narrowed almost to occlusion 1.5 cm from the orifice. There was irregular thickening of the walls of the descending and circumflex branches with some calcification. The right coronary artery exhibited some thickening of the wall irregularly, but the lumen was of good size and free. The myocardium was thickened—the left ventricular wall measuring 1.9 cm—but there was no gross or microscopic fibrosis. In figure 7 A a frozen section of the left coronary artery shows at the right an elongated ovoid region staining deeply with hematoxylin. This was a focus of calcification, the calcium crystals taking a deep stain with hematoxylin. The amount of material taking the fat stain is small. In figure 7 B a neighboring unstained section is seen under polarized light. The doubly refractive material is abundant and is for the most part made up of solid crystals of cholesterol, which do not stain with fat stains. In the inner layers of the intima below the lumen are some finely granular, doubly refractive bodies. When pressure was brought on the warmed section at this region there was freed into the lumen the material shown in figure 7 C.

This coronary artery illustrates a chronic fibrotic process, with solid cholesterol crystals, which has gone on to necrosis and calcification but still shows active cholesterophages in the internal portion of the intima.

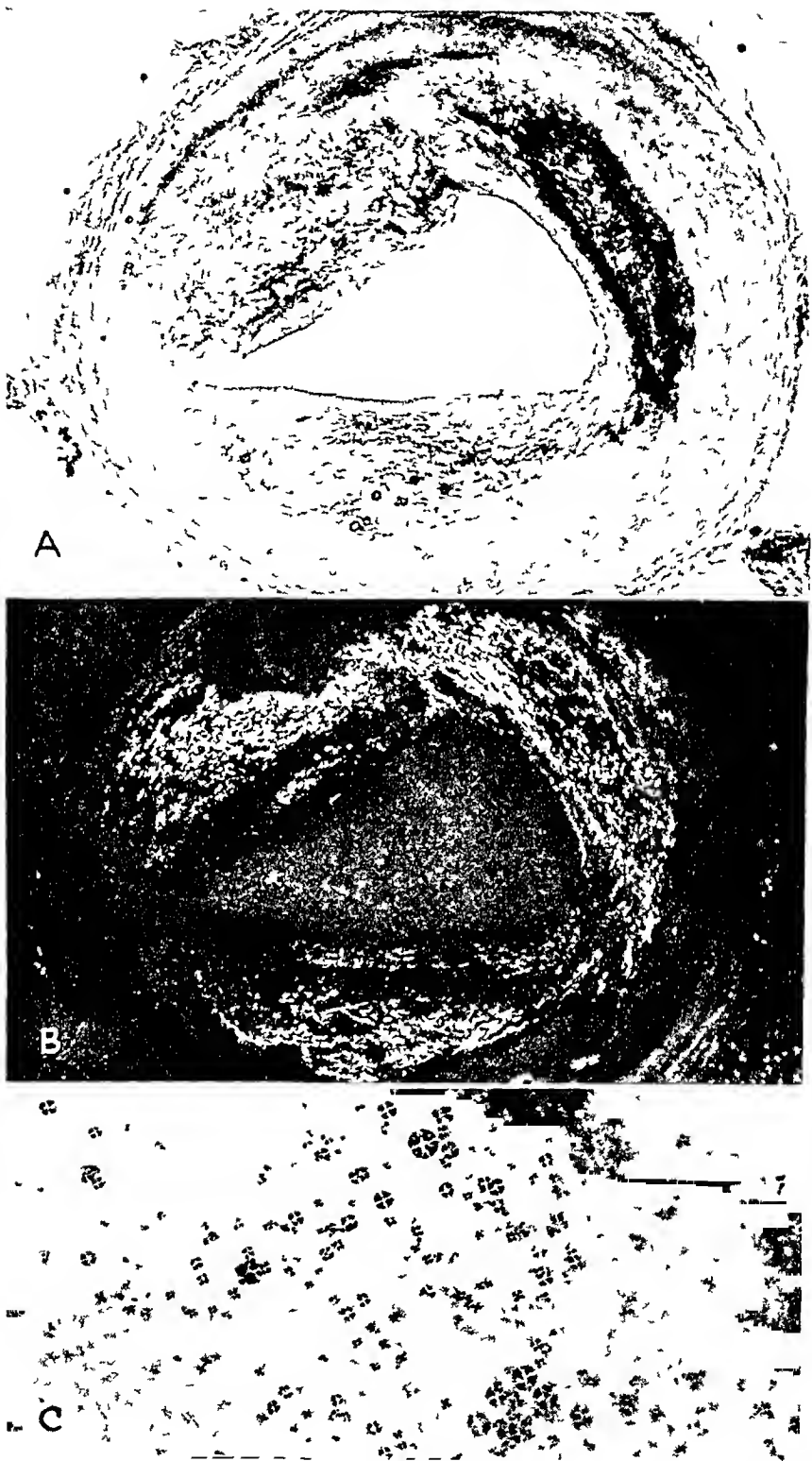


Fig 7—*A*, frozen section of a human coronary artery. The long ovoid, deeply staining region at the right is a focus of calcification. Hematoxylin and sudan IV,  $\times 35$

*B*, same section unstained, under polarized light  $\times 35$

*C*, cholesterol ester crystals expressed from an active portion of the lesion in the lower part of the intima near the lumen. The freed crystals are in the lumen under polarized light  $\times 200$



CASE 4—L. F., a short stout man, measuring 5 feet 2½ inches (158.5 cm) and weighing 173 pounds (78.5 Kg), was found in church, unconscious. He was admitted to a hospital with right hemiparesis and died within four hours. The heart weighed 525 Gm, and there was slight old thickening of the anterior flap of the mitral valve. In the left coronary artery, particularly in the descending branch, there was patchy high grade sclerosis and narrowing of the lumen, usually eccentric. The right coronary artery was tortuous, with a thick opaque yellow inner layer almost continuous. There was no gross (or microscopic) evidence of fibrosis of the hypertrophied myocardium (thickness of the left ventricular wall, 2 cm). Death was due to a hemorrhage of the left cerebral hemisphere.

A section of a lesion in the descending branch of the left coronary artery discloses evidence of the cumulative character of the atherosclerotic lesions (fig. 8)



Fig. 8—Human coronary artery in which a series of cumulative lesions are narrowing the lumen. Zenker solution fixation, Mallory's aniline blue method,  $\times 40$ .

There is a series of at least six apparently distinct foci, each of which probably represents a period of excessive ingestion of cholesterol. There remain between the hyaline scarred foci some cholesterophages in places, and an active process is taking place in the inner part of the thickened intima, notably at the lower end. Lesion 1 shows organization with vascularization from vasa vasorum, which is unusual in coronary lesions except after thrombosis. Lesions occurring before thrombosis tend to be vascularized by capillaries from the coronary lumen.

CASE 5—J. B., 72 years of age, was struck by an automobile and was dead on arrival at the hospital. Death was due to multiple injuries. The heart weighed 600 Gm. The wall of the left ventricle measured 1.4 cm, the right ventricle, 0.7 cm. The muscle, firm, medium red, showed no fibrosis. The cavities were dilated, the valves, essentially normal. The left coronary artery was occluded near the orifice.

In the anterior descending branch the wall was thickened and calcified discontinuously but there was a small free lumen. In the circumflex branch there was moderate thickening of the wall with a free lumen. The right coronary artery, of large size, had a large free lumen.

In figure 9 *A* a frozen section of the descending branch of the left coronary artery discloses eccentric fibrosis and, invading the wall, cholesterophages which

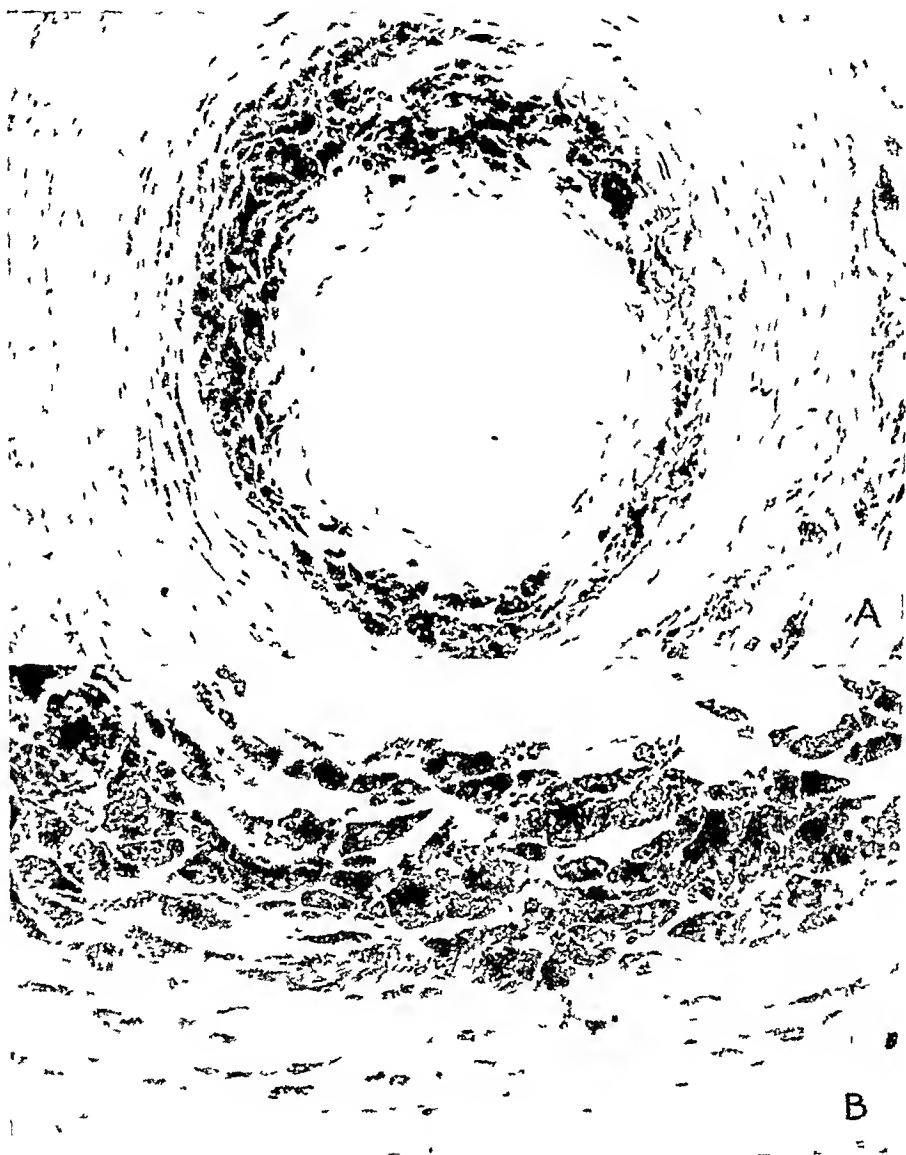


Fig 9—*A*, human coronary artery in which an old fibrous lesion is being invaded by cholesterophages. Frozen section, hematoxylin and sudan IV,  $\times 50$

*B*, detail of cholesterophages from lesion shown in *A*, under enlargement  $\times 200$

he in characteristic irregular ameboid shapes in the fibrous tissue of the wall. Figure 9 *B* brings out in enlargement the finely granular character of the fat-staining contents of the cells. Outside the ring of cholesterophages there are elongated cells whose contents stain with sudan IV. Under polarized light the contents of the cholesterophages consist of crystalline ester cholesterol. The con-

tents of the elongated cells are not doubly refractive. These cells appear to be fibroblasts which have taken over crystalline ester cholesterol from cholesterophages of an earlier invasion and have dissolved the crystals in the process of removing them.

This coronary artery reveals that active invasion of the arterial intima may occur at advanced ages and is not restrained by chronic fibrosis of the intima.

For examples of coronary lesions which were the primary cause of death in cases of sudden death the reader is referred to an earlier publication<sup>6c</sup>. These lesions were from persons who were not hospitalized before death, and show the coronary process without secondary



Fig 10—Aortic ring, ascending aorta and orifice of the innominate artery of a cholesterol-fed rabbit, showing plaques of lime salts (see text)

inflammatory and repair changes arising during a period of hospital care. The lesions in fatal cases tend to be advanced and may not show any crystalline cholesterol. This type of lesion is best understood after studies of the progressive lesions that precede the fatal termination.

In answer to the perennial question "Does calcification occur in the lesions of the rabbit fed cholesterol?" figure 10 is presented. This is a photograph of the aortic ring, the ascending aorta and the innominate orifice of a rabbit fed cholesterol over a period of ten months and allowed to live for one year following the cessation of cholesterol feeding. The lesions in general have healed with fibrosis thickening of the vessel wall and increase of elastica following the removal of

most of the cholesterol. The typical calcified plaques, concave to conform to the curved surface of the aorta, persist and vary from small to large masses. This aorta illustrates the fact that atherosclerosis is a curable disease in the rabbit, as it is in man, with monumental deposits of calcium marking the sites of the more ugly foci of the disease, that went on to necrosis.

#### COMMENT

The illustrations speak for themselves. They picture actual conditions which were found in human lesions and in those of the rabbit fed cholesterol. The studies under polarized light bring out the sites of crystalline ester cholesterol deposits and make it possible to follow the transport of the material and the relation of this to the lesions of atherosclerosis without the use of isotopes. One can actually see and demonstrate these things visually without too great dependence on inferences which may be speculative.

Aschoff's doubt as to whether the relation of cholesterol to the disease was causal or casual has been dissipated by the evidence that crystalline ester cholesterol is an irritant, comparable to silica. Studies of early human and rabbit lesions of atherosclerosis have made plain that injury to the intima is not a necessary factor in the causation of the disease, though there is evidence that injury may favor local manifestations. Wear and tear conditions tend to be general in their effect on arteries, while atherosclerosis is a focal disease. However, it may produce diffuse effects by the union of lesions as in the massive atherocheumas met with in the lower part of the abdominal aorta. Wear and tear tend to produce diffuse dilatation with thickening of the wall due to fibrosis, or with thinning of the wall due to weakness.

Hueper, in a series of papers, has described the results produced by introducing into the animal body certain inert substances, macromolecular in character, with molecular weights of over 1,000. The substances used in his studies included polyvinyl alcohol, methyl cellulose, acacia, pectin and others. Characteristic of these substances is the fact that on being introduced into animals, in massive amounts, particularly by the intravenous route, they produce what is called the "macromolecular hematologic syndrome," which is marked by loss of hemoglobin, of fibrinogen, of plasma protein, and is associated with thrombopenia or thrombocytosis, leukopenia or leukocytosis and a hemorrhagic diathesis, etc. Degenerative vascular changes, often resembling or identical with atheromatous or arteriosclerotic lesions are found in the majority of the macromoleculoses, according to Hueper<sup>19</sup>. He stated that his observations are reported "not only because they open a new rapid method for the experimental production of atheroma in animals but because they furnish important evidence concerning the formal and

the causal genesis of atheromatosis and arteriosclerosis<sup>20</sup> Discussion of these claims is the purpose of the present comment

The most striking changes in the vascular system were obtained by injecting colloidal suspensions of polyvinyl alcohol into dogs, and for that reason those experiments form the basis for the present discussion. Polyvinyl alcohol is a plastic, produced by the Dupont Company, and recommended commercially as an inert substance that forms protective films which are impervious to greases, to many solvents and to many gases. This material was injected into animals in a 5 per cent colloidal solution in isotonic sodium chloride solution. In Hueper's studies 8 dogs received varying amounts of this solution, 4 by the intravenous route and 4 by intraperitoneal injection. Of the animals given intravenous injections, 1 dog, receiving 285 cc of the solution, was moribund on the sixth day and was killed. A second received 630 cc and was killed on the fourteenth day. A third received 1,145 cc and died on the twenty-fourth day. The fourth received 915 cc and died on the twenty-fourth day. Of the dogs receiving the fluid intraperitoneally 1 died on the twenty-first day following the introduction of 2,725 cc of the solution. As was to be expected, the other dogs, receiving smaller amounts (490, 490 and 695 cc), survived for longer periods than the intravenous group, and were killed on the ninety-second, twenty-third and thirty-third days, respectively.

In the histologic description of the lesions found in the bodies of these animals no distinction is made between the lesions of the dogs given the fluid intravenously and those of the dogs receiving the fluid intraperitoneally. I have assumed that the most striking vascular lesions described and illustrated in Hueper's studies appeared in the dogs in which the polyvinyl alcohol was introduced by intravenous injection.

The vascular lesions consisted of balloon-like swellings of the endothelial cells which lined the inner aortic wall in a single or in several layers and had the character of foam cells. In more advanced lesions there was a narrow accumulation of foamy histiocytes between the endothelium and the media. Still older lesions exhibited a marked cushion-like thickening of the intima consisting of foam cells and occasionally a small capillary vessel. "The topographic relation of these foam cells to the endothelial lining, from which they seemed to extend, made it probable that these histiocytic cells were derived from the endothelial cells"<sup>19</sup> In the liver the cell cords were narrow and composed of atrophic liver cells, pushed apart by proliferated and markedly swollen Kupffer cells. The contents of the foam and Kupffer cells were made up of polyvinyl alcohol—unchanged.

The necessarily fatal result of the intravenous injection of large amounts of this material is obvious. A substance recommended com-

20 Hueper, W C Arch Path 33 1, 1942

mercially because of its inertness is injected intravenously into animals and may be retained in the circulating blood for long periods because it cannot be metabolized. Furthermore, this substance is introduced in overwhelming dosage. It is estimated by Hueper that in some instances up to 75 per cent of the fluid portions of the blood consisted of this foreign body.<sup>19</sup> The scavengers ordinarily employed in removing foreign bodies from the blood—the Kupffer cells—are manifestly incapable of taking care of the problem, and the vascular endothelium is called into action in a fruitless effort to meet the emergency. Disruption of all functions and death of the animals is the inevitable result under the dosage used.

Hueper<sup>20</sup> also carried out "chronic experiments" in dogs and rabbits with methyl cellulose, also a plastic, in 2 per cent solution in isotonic sodium chloride solution, some animals living fifty-seven to ninety days. The vascular lesions resembled those described in the polyvinyl experiments, but were less extensive. With reference to the dosage of methyl cellulose he reported "It must be emphasized that the various organic lesions mentioned are produced only when highly excessive amounts of methyl cellulose are introduced into the animal organism."<sup>20</sup>

In discussing the relation of macromolecular vascular lesions with those of atherosclerosis it is well to remember that the cholesterol in human atherosclerosis is not introduced intravenously. It is true that cholesterol is a polymer and a macromolecular substance. It differs from the substances studied by Hueper in that it is found in the animal body and is not inert. Though little is known about its metabolism within the animal body, one does know that it is metabolized. In whatever form it is ingested it apparently is converted by metabolism into the crystalline ester form before it becomes capable of causing atherosclerosis. It is one of the essential substances of animal life. In contrast, the macromolecular substances studied by Hueper are truly foreign bodies. The intravenous method of introducing them into animals should cause the lesions which arise to be diffuse, while atherosclerotic lesions are focal, and selectively located. The vascular macromolecular lesions are produced rapidly, as is true in foreign body experiments, in contrast to the long latent period in the cholesterol-fed rabbit before arterial lesions appear. The choice of experimental animals is a matter of indifference with the macromolecules, as would be expected in pure foreign body experiments. Indeed, the most impressive lesions were obtained in the dog, an animal in which atherosclerosis has been produced only after disabling the thyroid gland with thiouracil. The lesions in the macromolecules apparently represent a pure foreign body reaction provoked by inert substances, and carried to an unusual degree because of the tremendous dosage used. They are not reproductions, but are at best simulacra, of the lesions of human and experimental atherosclerosis.

Moreton<sup>21</sup> reported that by dark field illumination of plasma large colloid lipid particles could be observed and photographed as bright points of light. In normal plasma no particles could be seen, apparently because of their small size. Under centrifugation the large particles could be concentrated in the upper layers of the plasma. They were present in the plasma of continued hyperlipemia and appeared in normal plasma three to five hours after a meal rich in animal or vegetable fat. Moreton accepts Hueper's theory of causation of atheroma. To quote him:

the primary factor and *sine qua non* in arteriosclerosis is the presence in the circulating blood of coarsely suspended colloidal particles considerably larger than those found in normal plasma and composed of or containing, a substance relatively resistant to the resorptive and removal mechanisms of the arterial intima. present evidence indicates that the ingestion of fat-rich meals, by producing the temporary appearance of large lipid particles in the blood, causes the normal defense mechanisms of the intima to retain some of these particles and thus gradually and infinitesimally to build up the full picture of stenotic and occlusive arterial disease.

The objections to Moreton's thesis are (a) the failure of Hueper's procedure to reproduce the lesions of human and rabbit atherosclerosis and (b) the absence of evidence that the mere presence of large lipid particles in the plasma is adequate to produce the selectively focal lesions of human or rabbit atherosclerosis.

#### SUMMARY

Visual evidence is presented which, added to the data already published, indicates that atherosclerosis is a metabolic disease, that the relation of crystalline ester cholesterol to the lesions is as specific as is that of the tubercle bacillus to tuberculosis, that the focal character of the disease and the localization of its lesions are due to the crystalline esterification of excess cholesterol (in whatever form it is ingested) in the liver and the scavenger action of Kupffer cells in removing that excess from liver cells, transporting it into the circulation and depositing it in the subendothelial layer of the arterial intima in regions where the cholesterophages can make contact with the arterial intima.

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21 Moreton, J. R. Science **106** 190, 1947, **107** 371, 1948

# MULTINUCLEATED EPITHELIAL CELLS IN THE TUBULES OF THE HUMAN KIDNEY

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MULTINUCLEATED giant epithelial cells occurring in the renal tubules have elicited the attention of many investigators, who most often observed them incidentally during the study of other renal lesions. They were described originally by Weigert,<sup>1</sup> who noted them in a contracted kidney. Subsequent students of human pathology recorded their presence in association with eclampsia,<sup>2</sup> infarct,<sup>3</sup> chronic acid poisoning,<sup>4</sup> glomerulonephritis,<sup>5</sup> nephrosclerosis,<sup>6</sup> and mercury bichloride intoxication.<sup>7</sup> In a comprehensive study of regenerative processes in damaged human kidneys Tulp<sup>8</sup> found them in many conditions, whereas Wittich<sup>9</sup> in an analysis of 150 consecutive autopsies carefully characterized them and enumerated their incidence in 33 cases of widely varied causations. The last-named author agrees with previous interpreters that the cells represent abnormal forms of regeneration.

Other workers have been able to produce similar cells in the kidneys of animals by various experimental procedures. Podwyszoński,<sup>10</sup> a pioneer in the study of epithelial regeneration, observed them in kidneys which had been subjected to mechanical trauma, and his observation was substantiated by Ribbert.<sup>11</sup> These forms were produced in animals by

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1 Weigert, C. *Samml. klin. Vortr.*, 1878, no. 162-163 (*Inn. Med.* no. 2), p. 1411.

2 Prutz, W. *Ztschr. f. Geburtsh. u. Gynäk.* **23**, 1, 1892. Leusden, P. *Virchows Arch. f. path. Anat.* **142**, 1, 1895. Ellerman, V. *ibid.* **171**, 557, 1903.

3 (a) Thorel, C. *Virchows Arch. f. path. Anat.* **146**, 297, 1896. (b) Foa, P. *Beitr. z. path. Anat. u. z. allg. Path.* **5**, 275, 1889.

4 Thorel, C. *Deutsches Arch. f. klin. Med.* **84**, 173, 1905.

5 Rossle, R. *Virchows Arch. f. path. Anat.* **170**, 375, 1902. Oertel, H. *Publication of the Russell Sage Institute of Pathology*, Philadelphia, W. B. Saunders Company, 1909, vol. 1.

6 Herxheimer, G. *Beitr. z. path. Anat. u. z. allg. Path.* **45**, 253, 1909.

7 Heinecke, A. *Beitr. z. path. Anat. u. z. allg. Path.* **45**, 197, 1909.

8 Tulp, A. *Ueber die Regenerationsvorgänge in den Nieren des Menschen*, Jena, Gustav Fischer, 1912.

9 Wittich, W. *Virchows Arch. f. path. Anat.* **206**, 341, 1911.

10 Podwyszoński, W., Jr. *Beitr. z. path. Anat. u. z. allg. Path.* **2**, 1, 1888.

11 Ribbert, H. *Arch. f. Entw. u. Mechn. d. Organ.* **18**, 207, 1904.



poisoning with uranium<sup>12</sup> and by restricting them to a diet containing excess inorganic phosphates<sup>13</sup> More recently, in animals fed diets extremely deficient in potassium, lesions and multinucleated cells developed identical with those produced by ingestion of excess phosphates<sup>14</sup> The formation of giant cells was noted in chloride-deficient rats by Lowenhaupt and Greenberg,<sup>15</sup> although in these animals the cells were in the distal rather than the proximal convoluted tubules

Because of this recent suggestive influence of diet on the structure of renal tubules and the frequency of the giant epithelial cells in routine autopsy material, it was decided to investigate the incidence of these abnormal cells The facile appellation "atypical regeneration" designates a theory of origin but fails to elucidate their pathogenesis with accuracy, for they may not represent abnormal regeneration in all instances but may be a type of degeneration in some cases, as well as a failure of regeneration Lubarsch<sup>16</sup> suggested that they are "irritation cells," but he neither specified the irritant nor indicated whether the hypothetical irritant initiated regeneration or degeneration

#### MATERIAL STUDIED

The kidneys of 256 recent consecutive subjects of autopsies were examined both grossly and microscopically Representative portions of the organs were fixed in formaldehyde solution U S P diluted 1 in 10, embedded in paraffin, sectioned at 8 microns and stained with hematoxylin and eosin Occasional sections were cut to a depth of 15 microns and stained with safranin and trinitrophenol for the study of cell contour and mitotic figures The frequency of multinucleated cells was graded as 1, 2 and 3 plus when they occurred 1 per several low power fields, up to 10 per low power field, and more than 10 per field, respectively With this arbitrary division, they fell into clearly defined groups Attention was directed to the multiplicity of nuclei of the average giant cell by a count of 100 random multinucleated cells in each case Correlative data concerning age, sex and incidental disease were accumulated from the protocols

#### RESULTS

Among the 256 autopsies reviewed there were 39 in which multinucleated epithelial giant cells of varying degree of incidence were noted, representing 15.2 per cent of the total number of autopsies The cells were found exclusively in the proximal convoluted tubules and were not observed in the lower nephron It was not possible to determine their location in any particular segment of the proximal tubules, which appeared almost uniformly involved throughout their length in the more extensive lesions In our series the tubules containing giant cells were not situated in relation to specific disease areas, such as scars, infarcts and inflam-

12 Oliver, J J *Exper Med* **21** 425, 1915

13 MacKay, E M, and Oliver, J J *Exper Med* **61** 319, 1935

14 Follis, R H, Orent-Keiles, E, and McCollum, E V *Am J Path* **18**:29, 1942

15 Lowenhaupt, E, and Greenberg, D M *Arch Path* **42** 49, 1946

16 Lubarsch, O *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol 6, pt 1, pp 584-585

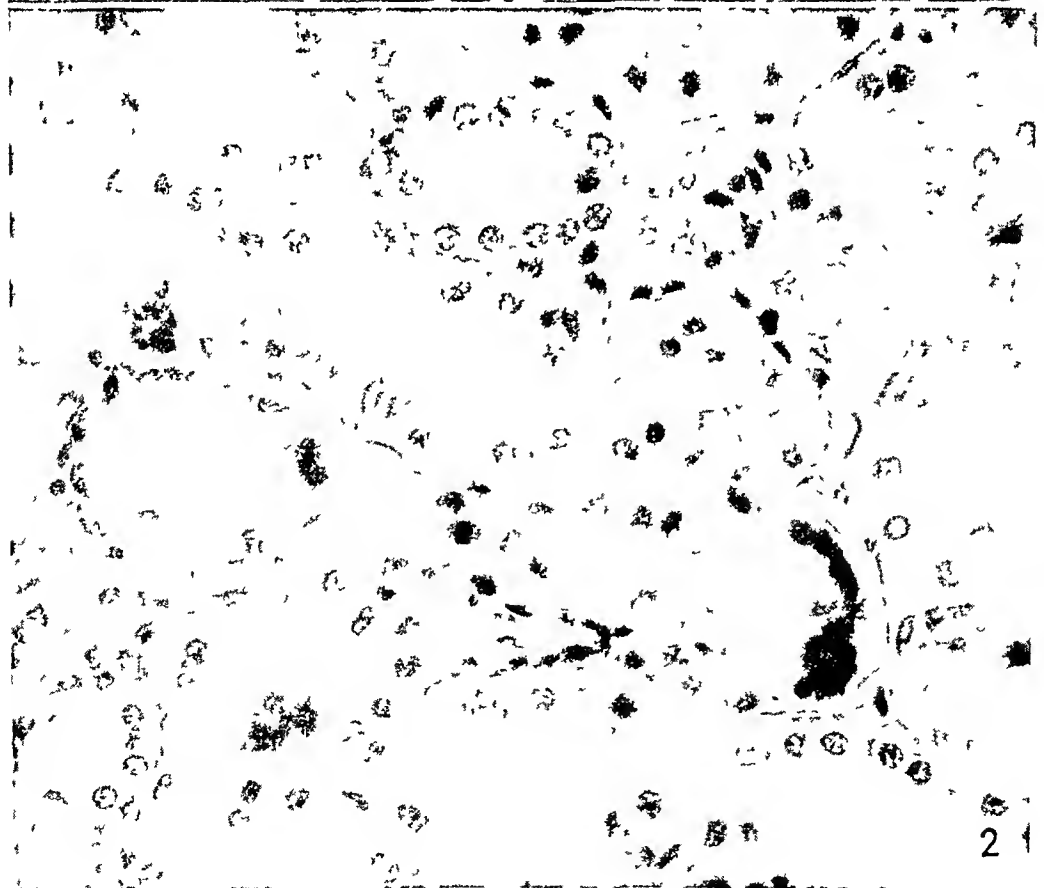
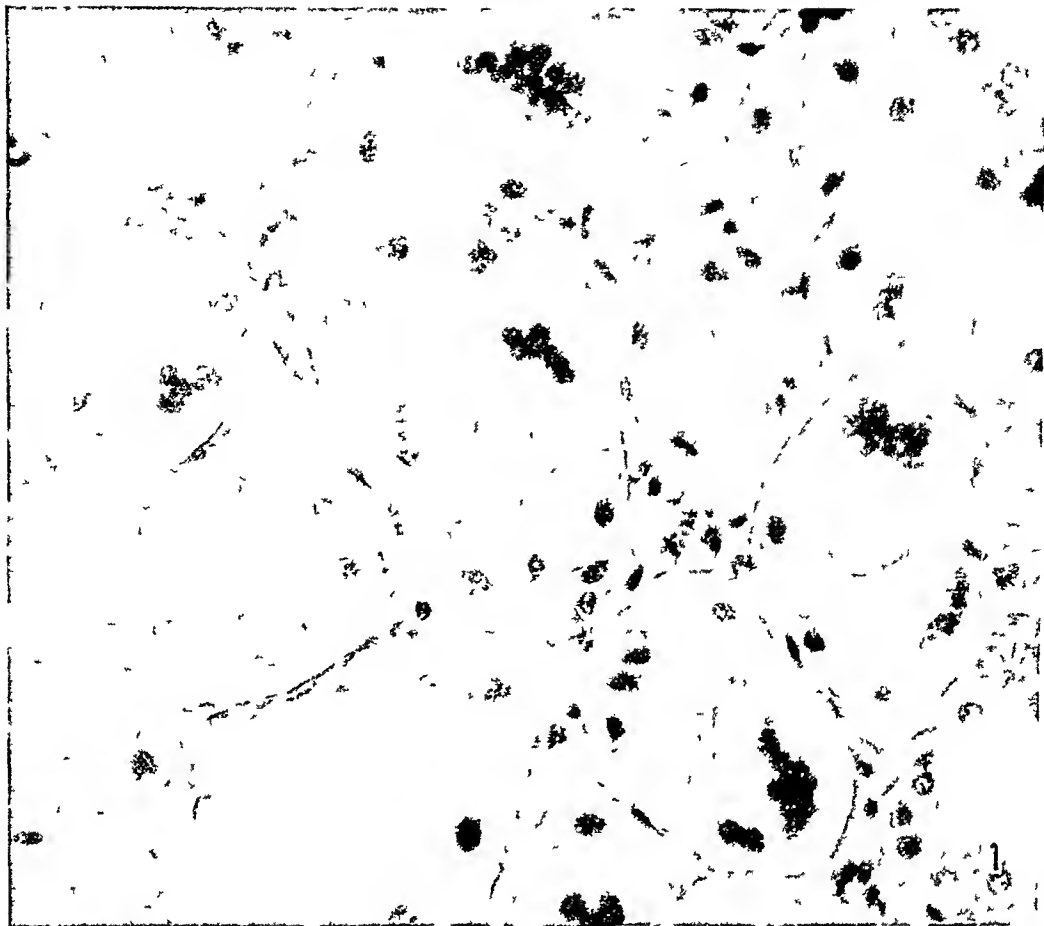


Fig 1—The parabasilar clumping of the nuclei of the giant cells is apparent. There is disparity of size in the nuclei. Hematoxylin and eosin,  $\times 420$ .

Fig 2—The clumping of nuclei and their linear arrangement are evident in some cells. There is a homogeneous cytoplasm in the multinuclear cells. Hematoxylin and eosin,  $\times 420$ .

mation, but were scattered at random throughout the section. Such tubules were neither degenerated nor hypertrophied and generally resembled the normal tubules.

The multinucleated cells usually formed a portion of the wall of the tubule, with displacement of an area equivalent to less than 4 normal cells. Rarely they appeared unattached within the lumen. In contour the forms varied, some were broad and projecting (75 to 100 microns), others flat and narrow (figs 1 and 2). Basement membranes were clearly defined, whereas the free border was uneven and indefinite and infrequently was distinguished by a brush border. The cytoplasm was dense, deeply eosinophilic and homogeneous, devoid of vacuoles and granularity. Fat was not demonstrable with sudan IV. The number of nuclei varied widely, ranging from 4 to 30, and was not strictly proportional to the size of the cell, the commonest number was 5 to 6. It was, however, noticed that the number of nuclei bore a rough proportionality to the incidence, and as it increased so did the average of the nuclei per cell. Most nuclei were smaller than normal and sometimes appeared even as minute particles, 2 to 3 microns in diameter, within any cell the range of size might be considerable, usually with minute forms.

TABLE 1—*Incidence of Renal Giant Cells in Relation to Diseases of the Kidney*

| Disease                    | Cases in Which Microscopic Sections Showed Given Grade of Involvement * |    |    |       |
|----------------------------|---|----|----|-------|
|                            | 1+  | 2+ | 3+ | Total |
| Nephrosclerosis            | 7   | 8  | 2  | 17    |
| Pyelonephritis (chronic)   | 1   | 2  | 2  | 5     |
| Polycystic kidney          | 0   | 2  | 1  | 3     |
| Infarcts                   | 1   | 2  | 0  | 3     |
| Hemoglobinuric nephrosis † | 3   | 1  | 0  | 4     |
| No lesion                  | 3   | 4  | 1  | 8     |
| Total                      | 15  | 19 | 6  | 40    |

\* 1+ indicates 1 multinucleated giant cell per several low power fields, 2+, up to 10 cells per low power field, 3+, more than 10 per field.

† The hemoglobinuric nephrosis was incidental and not a direct cause of death.

predominant. They were heaped up irregularly, with a tendency to concentrate against the basement membrane, and, because of the hyperchromatic density, assumed a characteristic grouped appearance. The size, the density of packing and the intensity of staining obscured nuclear detail, although nucleoli might be observed in the larger, lighter members. Mitotic figures were absent in all cases.

The 39 cases in which the kidneys contained giant cells had no common pattern of disease and included such causes of death as carcinoma (10), coronary occlusion and cerebrovascular accident (6), tuberculosis, pneumonia and arterial thromboses (2), arteriosclerosis (4) and, in a single instance each, cirrhosis, endometritis, fat embolism, rheumatic fever, polycystic kidney and malignant lymphoma, in 1 case the cause remained undetermined. In no case was any history of renal insufficiency detected, so that neither the dominant disease process nor the assessment of renal function was clinically indicative of the occurrence of these cells. The high incidence in cases of carcinoma, coronary disease and cerebrovascular accident is noteworthy.

On the other hand, when the degrees of the giant cell involvement were correlated with specific disease processes in the kidneys, it emerged (table 1) that in such kidneys as were affected by generalized and uniform sclerosis the incidence was highest and most extensive. It is also remarkable that there were no examples of glomerulonephritis of any type, although fibrosis and shrinkage

were often prominent in these cases. The small number of kidneys with infarcts is also contrary to expectation. But perhaps most striking is the group in which no definite renal lesions were seen and yet the distribution of giant cells was extensive, indicating that though sclerosis might be an important concomitant, it was not solely a determining factor in the causation of these cells. Because of the close association which certain authors believe exists between chronic glomerulonephritis and such giant cells, the slides from all autopsies of persons with this disease over a ten year period were examined to establish this relationship. In only 2 of the cases was there an incidence of giant epithelial cells of 1 plus. A similar study of renal infarcts revealed no peculiar high or severe occurrence of the cells in the tubules adjacent to or between infarcted areas. It is therefore apparent that epithelial giant cells are not specifically related to any particular systemic disease, are often found in otherwise healthy kidneys but are most commonly observed in sclerotic kidneys.

The relationship of the giant cells to age is more evident. When the incidence is taken as a percentage of the number of cases examined in a particular decade (table 2), it is manifest that the proportion found in persons under 40 years of age

TABLE 2—*Incidence of Renal Giant Cells in Relation to Age*

| Age   | Cases | Cases with<br>Giant Cells | Percentage |
|-------|-------|---------------------------|------------|
| 0 10  | 48    | 1                         | 2.3        |
| 11 20 | 16    | 0                         | 0.0        |
| 21 30 | 16    | 1                         | 6.2        |
| 31 40 | 22    | 0                         | 0.0        |
| 41 50 | 34    | 5                         | 14.7       |
| 51 60 | 48    | 10                        | 20.8       |
| 61 70 | 41    | 11                        | 26.9       |
| 71 80 | 28    | 10                        | 35.7       |
| 81 90 | 8     | 1                         | 12.5       |
| Total | 256   | 39                        | 15.2       |

is negligible, although over one third of the autopsies studied fell into this age stratum. With increasing age, on the contrary, a steady rise is seen, which reaches a maximum of 35.7 per cent in the seventh decade. This sharp cleavage between the two age strata, above and below 40 years, indicates that age is an important factor in the formation of the epithelial giant cells, the incidence is 0.9 per cent in the former, 14.4 per cent in the latter. Although the incidence of the giant cells is closely related to age, the extent of occurrence is not. When severity of incidence is plotted against age, the distribution is not statistically different from one decade to another above the age of 40, so that one may regard age as having little effect on severity. Sex may also be excluded in our series, because, although more males were affected, in a ratio of 27:12, the distribution followed the proportion of males to females submitted to autopsies in this institution over a seventeen year period, which is 2,658:1,465. However it is noteworthy that 40.7 per cent of males had a 1 plus incidence, compared with only 25 per cent of females, owing to this disproportion, the influence of sex cannot be entirely dismissed in relation to severity.

#### COMMENT

The multinucleated giant cells found in the proximal convoluted tubules are not to be confounded with those occurring in the distal tubules. The latter are seen in various forms of lower nephron nephrosis

and have been described in multiple myeloma,<sup>17</sup> sulfonamide reactions<sup>18</sup> and hemoglobinuric nephrosis<sup>19</sup> Lowenhaupt<sup>20</sup> induced similar cell formation in the distal tubules by feeding rats a chloride-deficient diet, the cells were reacting to intraluminal deposits of calcium. In all such instances the cells represent a type of reaction to material deposited in the tubules, whether it be protein, salts or an organic concretion. On the other hand, the giant cell masses of the proximal tubules are not usually associated with a visible precipitate or concretion, although in mercury poisoning calcium may be deposited in the necrotic cells.<sup>21</sup> The distinction between these forms is therefore clearcut.

The sporadic observations that these cells are incidental to isolated lesions, together with the description of them given by Podwyszoński<sup>10</sup> in his studies of regeneration, induced most authors to accept them as a form of atypical regeneration, of these Thorel<sup>3a</sup> is a chief protagonist. This view was greatly strengthened by the careful study of Tilp on regeneration in the human kidney, in which he noticed their frequent occurrence and supported Thorel's explanation. Perusal of his monograph reveals, however, that he did not always distinguish between large cells and multinucleated giant cells, so that some cases remain doubtful, and further his interest was not so directed that he studied otherwise normal kidneys as controls. If one accepts all his cells as multinucleated forms, the incidence for his series is 33 per cent, with due regard for the precautions suggested this corresponds well with Wittich's observation of 33 instances from 150 consecutive autopsies, or 22 per cent. The inconsistency that they are seen with about equal frequency in a series of uncontrolled diseased kidneys and in kidneys selected at random hardly justifies the conclusion reached by Tilp that they represent a peculiar form of regeneration. Closer study of Wittich's paper elicits the fact that in 21, or just under 66 per cent, of his cases the only renal lesion is a parenchymatous degeneration, a condition often as dependent on postmortem as on antemortem events. This absence of a specific destructive lesion in such a high proportion of cases suggests that the stimulus to giant cell formation is not local and opens the issue to another explanation. It may be granted that under experimental conditions specific stimuli, such as cutting, freezing, injection of toxins and dietetic imbalance, can induce formation of these cells and that they are seen when the tissue is recovering from injury. However, it is equally to be conceded that with few exceptions such traumas are uncommon in human

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17 Bell, E. T. *Am J Path* 9: 393, 1933. Morison, J. E. *J Path & Bact* 53: 403, 1941.

18 More, R. H., McMillan, G. C., and Duff, G. L. *Am J Path* 22: 703, 1946.

19 Harman, J. W. *Irish J M Sc* 259: 480, 1947.

20 Lowenhaupt, E. *Arch Path* 42: 572, 1946.

21 Edwards, J. G. *Am J Path* 18: 1011, 1942.

kidneys. Moreover, we find that in conditions in which regeneration is most expected to participate, it is not especially found associated with these forms, we find no special incidence in either glomerulonephritis or infarct, as Wittich also noticed for nephritis and Rossle for infarcts. On the other hand, the uncommonly high incidence of the cells in shrunken kidneys and kidneys without other lesion indicates that we can seek the cause elsewhere than in distorted regeneration.

Two facts are salient. One is that no one has ever observed mitotic figures in these cells. Wittich is very specific on this point, and we have failed to find them in any of our cases with the safranin stain. Krompecher<sup>22</sup> in his classic study described mitoses in several types of giant cells, with careful illustration, and regarded this as a progressive development of the cell. On the contrary, he designated amitosis as a retrogressive change in this cell, a form of fragmentation of the nucleus or a degeneration. The absence of mitoses in the human renal giant cells and the fragmentary character of many of the nuclei suggest that they conform to the degenerative type of cell and are not a progressive, atypical form of hyperplasia. It is perhaps too extreme to assume, as did von Rath,<sup>23</sup> that once a cell divides amitotically it is doomed, but it is consistent to regard such a cell as a degenerative form, especially when it fails to effect cytoplasmic division as well and occurs in the midst of cells which divide usually by mitoses. The view that mitotic figures were present but vanished by the time of autopsy is not substantiated, because they often can be demonstrated for some time after death,<sup>24</sup> though chromosomal details are less clear. It is apparent, therefore, that the cells conform more closely to degenerative rather than progressive types.

The second fact is the obvious connection with age. Our findings are so striking that we reexamined the protocols of Tilp and Wittich, to obtain strong confirmation from their figures. Even though they gave no special attention to age as a factor, Tilp was drawn to remark, "Therefore it seems that the higher age group is most affected, as the cases of 81, 80, and 74 year old men show, so that perhaps the manner of reparation is characteristic of the aged," and later stated that there occurs "in older individuals the formation of plasmodia and in younger of mitoses." Even though Wittich offered no reference to age, a compilation of his findings agrees with ours remarkably well. We are urged by the compatibility of these different observations to regard age as a most significant factor in the pathogenesis of the renal multinucleated epithelial giant cell. The fact that this cell occurs in the shrunken, arterio-

22 Krompecher, E. *Virchows Arch f path Anat* **142** 447, 1895

23 von Rath, O. *Zool Anz* **14** 331, 1891

24 Mallory, F. B. *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938

sclerotic kidney is probably fortuitous, since both are common in aged persons either together or alone. If shrinkage represents a stimulus, it might be expected more frequently in the shrunken form of glomerulonephritis, where it has no especial incidence. It is, therefore, probably a form of senile change peculiar to the kidney, and not a type of regeneration.

#### SUMMARY

Multinucleated giant epithelial cells are found in the proximal convoluted tubules of the kidney in 15.2 per cent of routine autopsies.

They are rare before the age of 40 and from that time increase progressively in incidence.

Because of the lack of mitoses, the nuclear fragmentation and the peculiar age incidence they are regarded as degenerative rather than atypical regenerative forms.

# QUANTITATIVE ANALYSIS OF THE GROWTH OF EPIDERMIS

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THURINGER'S<sup>1</sup> data on mitosis observed in the strata of the epidermis of the human scalp afford a means for making quantitative estimates of the relationships between the number of cells of the several strata and the rates of reproduction of the different cell types. The following analysis is based on the classic picture in which the basal cell layer is assumed to be the generating layer from which cells are transformed into spinous cells which in turn become granular and finally cornified cells. Cowdry and Thompson<sup>2</sup> have pointed out that the finding that cell division occurs in the spinous layer is not inconsistent with the concept that the basal layer is the generating layer. It will be assumed here that after a basal cell becomes a spinous cell it undergoes a series of mitotic divisions before it becomes a granular cell. In accordance with Thuringer's data it will be assumed that the granular cell does not undergo mitosis.

The purpose of the analysis is to indicate the manner in which the mitotic index can be applied to the different strata of the epidermis in order to estimate the time sequence of the stages of differentiation in the history of a cell. The time spent by a cell in each stage of differentiation, the rate at which cells regenerate in the active strata, the number of divisions a cell undergoes as a spinous cell and the total time of renewal of the entire epidermis will be computed. The analysis will serve to indicate the approximations which must be made at the present state of knowledge and also to point out the important role which the duration of the mitotic process and the intermitotic time play in any quantitative analysis.

In order to make use of Thuringer's data on the mitotic index of the epidermis of the scalp it is necessary first to count the relative numbers of cells in the strata designated by Thuringer, namely, the basal, the lower third of the spinous ( $l/3$ ), the middle third of the spinous ( $m/3$ ), the outer third of the spinous ( $o/3$ ) and the granular stratum. Fortunately, Thuringer<sup>1a</sup> has published excellent photomicrographs of the scalp. In table 1, first column, is the relative number ( $n$ ) of the cells in

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1 Thuringer, J. M. (a) *Anat. Rec.* **28** 31, 1924, (b) **40** 1, 1928

2 Cowdry, E. V., and Thompson, H. C. *Anat. Rec.* **88** 403, 1944



each stratum taken from Thuringer's photomicrographs. It should be pointed out that the enumeration was made of nuclei rather than of cells, since the nuclei are definitely discernible, whereas the cells are not. Also, it has been assumed that the tissue sections shown in Thuringer's photomicrographs were cut perpendicularly to the plane in which the basal cells lie. The numbers of spinous and granular cells relative to the number of basal cells seen in a section are dependent on the angle of cutting. The analysis based on the relative numbers of cells depends on the sectioning being perpendicular to the plane of the basal layer.

Table 1 shows the mitotic indexes of the strata computed from the values of  $n$  and Thuringer's published distribution of mitoses in the strata. If the average mitotic index for the entire epidermis is  $MI$  (which

TABLE 1—*Mitotic Indexes of the Strata of the Epidermis Based on Thuringer's Original Data<sup>1b</sup> and Photomicrographs<sup>1a</sup>*

| Cell Stratum  | Relative Number of Cells ( $n$ ) | Cells as Percentage of Total * | Percentage of Mitoses in Layer | Mitotic Index of Layer ( $m$ )† |
|---------------|----------------------------------|--------------------------------|--------------------------------|---------------------------------|
| Basal         | 10.0                             | 36.4                           | 12                             | $1.6 \times 10^{-4}$            |
| Spinous (1/3) | 8.1                              | 29.4                           | 30                             | $4.8 \times 10^{-4}$            |
| Spinous (m/3) | 5.2                              | 19.0                           | 46                             | $11.3 \times 10^{-4}$           |
| Spinous (o/3) | 4.2                              | 15.3                           | 12                             | $3.7 \times 10^{-4}$            |
| Total spinous | 17.5                             | 63.6                           | 88                             | $6.5 \times 10^{-4}$            |
| Granular      | 4.2                              |                                | 0                              |                                 |

\* The values are for mitotically active layers only.

† This was computed by equation 1.

Thuringer<sup>1b</sup> gave as  $4.15 \times 10^{-4}$  mitoses per cell), then the mitotic index for each layer ( $m$ ) is given by equation 1

$$m = \frac{\% \text{ of mitoses in the layer}}{\% \text{ of all cells in the layer}} \times MI \quad (1)$$

It should be noted that the total mitotic index  $MI = 4.15 \times 10^{-4}$  has not been corrected for the presence of the nonactive granular cells. They comprise 4.2 cells in a total of 31.7, which leads to a correction of 12.5 per cent, making the value of  $MI = 4.7 \times 10^{-4}$  which was used in computing the values of  $m$  in table 1.

In the application of the mitotic index one uses the fundamental relationship of equation 2, below, between the mitotic index,  $m$ , and the duration time of mitosis,  $T$ , and the cell doubling time,  $L$ , which is also the intermitotic time

$$m = T/L \quad (2)$$

For a discussion of this relationship I refer to a previous article.<sup>3</sup> The expression of the mitotic index in equation 2 is applicable to stationary

populations of cells—e g, populations in which there is continual turn-over but in which the total number of cells present remains constant with time In the analysis I shall use the subscripts b, s, and g to denote the basal, total spinous and granular strata, respectively For convenience, I shall introduce the regeneration constant,  $\lambda$ , which denotes the cells generated per cell per hour Thus if there are  $n_b$  basal cells, the rate of regeneration of basal cells will be  $n_b\lambda_b$  cells per hour The quantity  $\lambda$  is related to  $L$  as follows  $\lambda = 1/L = m/T$

In order to use the mitotic index given in equation 2, it is necessary to know the duration time of mitosis,  $T$  Thuringer<sup>1b</sup> made estimates of this time for human material and stated that it is of the order of  $1/4$  to  $1/2$  hour I shall compute my data using the two values, namely,  $T = 1/4$  hour, and  $T = 1/2$  hour Table 2 shows the values of  $\lambda$  and

TABLE 2—Regeneration Constants and Relative Regeneration Rates of the Strata of Epidermis

| Cell Stratum  | Relative Number of Cells ( $n$ ) | $T = 1/4$ Hour       |              | $T = 1/2$ Hour        |            |
|---------------|----------------------------------|----------------------|--------------|-----------------------|------------|
|               |                                  | $\lambda = m/T$      | $n\lambda$ * | $\lambda = m/T$       | $n\lambda$ |
| Basal         | 10.0                             | $6.4 \times 10^{-4}$ | 0.0064       | $3.2 \times 10^{-4}$  | 0.0032     |
| Spinous (1/3) | 8.1                              | $19 \times 10^{-4}$  | 0.0154       | $9.5 \times 10^{-4}$  | 0.0077     |
| Spinous (m/3) | 5.2                              | $45 \times 10^{-4}$  | 0.0234       | $22.5 \times 10^{-4}$ | 0.0117     |
| Spinous (o/3) | 4.2                              | $15 \times 10^{-4}$  | 0.0063       | $7.5 \times 10^{-4}$  | 0.0032     |
| Total spinous | 17.2                             | $26 \times 10^{-4}$  | 0.0455       | $13.5 \times 10^{-4}$ | 0.023      |

\*  $n\lambda$  = cells per hour, where  $\lambda$  = cells per cell per hour

$n\lambda$  when both values of  $T$  are used It then gives the number of cells generated per hour for the relative number of cells ( $n$ ) given in column 1

A comparison of the relative regeneration rates of the basal and the total spinous layer shows that approximately 8 cells are produced in the total spinous layer while 1 cell is produced in the basal layer by mitosis This estimate is based on the assumption that the division time,  $T$ , is the same in both layers The ratio of the relative regeneration rates is  $n_s\lambda_s/n_b\lambda_b = n_s(m)_s/n_b(m)_b$ , and from table 2, last column, this ratio is  $0.023/0.0032 = 7.2$  In a first approximation the ratio suggests that a cell undergoes three divisions while in the spinous state ( $2^3 = 8$ ) while a basal cell undergoes a single division

A more detailed analysis can be made by the use of the fundamental assumption that the tissue strata are in a state of cellular equilibrium According to his assumption, the rate at which cells are produced by mitosis in the basal layer equals the rate at which cells leave the basal layer to become spinous cells Similarly, the spinous cells leave the spinous state at a rate equal to the rate at which they are generated in the spinous layer If there are  $v$  divisions undergone by a cell after

it leaves the basal layer, the relative rate of generation of spinous cells,  $n_s\lambda_s$ , is equal to  $2^x$  times the relative rate of generation of basal cells,  $n_b\lambda_b$

$$n_s\lambda_s = n_b\lambda_b \times 2^x \quad (3)$$

In equilibrium the rate at which cells enter the granular layer is equal to the rate of production of spinous cells

$$n_g\lambda_g = n_s\lambda_s = n_b\lambda_b \times 2^x \quad (4)$$

In the case of the granular layer  $\lambda_g$  has no connotation of cell regeneration but is simply the reciprocal of the lifetime  $L_g$  of the cells in the granular state,  $\lambda_g = 1/L_g$ . The ratio of the relative numbers of spinous and basal cells becomes  $n_s/n_b = 2^x (\lambda_s/\lambda_b) = 2^x (L_b/L_s)$ , where the  $L$ 's are the intermitotic periods. From table 1, if  $n_b = 1$ , then  $n_s = 1.75$ . The intermitotic period,  $L_b$ , for the basal cells is computed as follows  $L_b = 1/\lambda_b = 1/3.2 \times 10^{-4} = 3,100$  hours (from table 2, where  $T = \frac{1}{2}$  hr). To solve for  $L_s$  then one has  $n_s/n_b = 1.75 = 2^x (L_b/L_s/3.1 \times 10^3)$  and  $L_s = 5.4 \times 10^3/2^x$ . Now  $L_s$  also equals  $T_s/(m)_s$ , so that it is possible to estimate  $T_s$  on the basis of the  $n$ 's and  $m$ 's instead of arbitrarily assuming a value as was done in table 2. Thus,  $T_s = (m)_s (5.4 \times 10^3)/2^x$ . From table 1  $(m)_s$  is  $6.5 \times 10^{-4}$  and therefore  $T_s = 3.5/2^x$ . If  $x = 3$ , then  $T_s = 0.43$  hour. This assumes that in the basal cells the duration of mitosis is  $T_b$ , or 0.5 hour, and that  $x = 3$ . The result means that the relative number of cells,  $n$ , and the mitotic activity indicate that the division time of the spinous layer is of the same magnitude as that of the basal layer. Conversely, the result indicates that if the duration times of mitosis of the two layers are carefully measured, it is possible to calculate the value of  $x$  from the  $n$ 's and  $m$ 's.

For an estimate of the lifetime of cells in the granular state one may refer to equation 4  $n_g\lambda_g = 2^x (n_b\lambda_b)$ . One takes  $x = 3$ ,  $n_b = 10$  and  $n_g = 4.2$  (from table 1) and  $\lambda_b = 3.2 \times 10^{-4}$  cells per cell hour (from table 2). Thus  $\lambda_g = 8 (10) (3.2 \times 10^{-4}) / 4.2 = 5.3 \times 10^{-3}$  per hour, from which the granular lifetime is  $L_g = 1/\lambda_g = 189$  hours or 7.9 days, if the value of  $T_b = \frac{1}{2}$  hour.

The detailed estimation of the time sequence of the spinous layer is not possible with the existing data on these cells. Only the broadest estimates based on crude assumptions can be made. Thuringer's arbitrary division of the spinous stratum into three layers with differing mitotic activities (table 1) gives an insight into the difficulties encountered. In the following discussion it will be assumed that the duration of mitosis is  $\frac{1}{2}$  hour. If no cell division occurred in the spinous layer, the lifetime of spinous cells would be given by the total number of spinous cells present divided by the rate of production of cells of the basal layer  $17.5/0.0032 = 5,500$  hours. But since it is known that division occurs in the spinous layer, this value of spinous lifetime is

too long On the basis of the mitotic index for the total spinous layer the intermitotic time is computed as  $L_s = 1/\lambda_s = 1/13.5 \times 10^{-4} = 740$  hours Similarly one can compute the intermitotic times in all three spinous layers as given in table 3 The sum of the intermitotic times in the three layers is 2,820 hours On the other hand, if one assumes the value of 740 hours per division and three divisions, the time spent in the spinous layer is 2,960 hours

A detailed analysis of the three layers of the spinous cells could best be made by carrying out the computations indicated by equation 3 and using first the equilibrium condition between the basal and lower third of the spinous layer But here, as in the equilibrium between the lower and the middle third of the spinous layer, the value of  $\lambda$  in equation 3 is unknown In order to specify more accurately the intermitotic times, the value of the duration of mitosis in the three sections of the spinous layer is required along with the mitotic indexes In

TABLE 3—Intermitotic Time,  $L$ , in Each Stratum of Epidermis\*

| Cell Stratum  | $T = \frac{1}{4}$ Hour<br>$L = 1/\lambda \uparrow$ | $T = \frac{1}{2}$ Hour<br>$L = 1/\lambda$ |
|---------------|--|---|
| Basal         | 1,550 hr   | 3,100 hr                                  |
| Spinous (1/3) | 525  | 1,050                                     |
| Spinous (m/3) | 220  | 440                                       |
| Spinous (o/3) | 665  | 1,330                                     |
| Total spinous | 370  | 740                                       |

\* The figures beyond the second place are not justified by the experimental data but are shown here for convenience of identification in the text

†  $\lambda$  is taken from table 2

table 3 the intermitotic times are computed on the assumption that in all cells the duration of mitosis is  $\frac{1}{2}$  or  $\frac{1}{4}$  hour, which must be regarded as a crude simplification to indicate the main trends of the times It is probable that at least the duration of mitosis of cells of the basal layer differs from that of cells of the spinous layer With this in mind one can make a speculative estimate of the time sequence of differentiation from table 3 by assuming  $T = \frac{1}{2}$  hour for basal cells and then assuming that  $T = \frac{1}{4}$  hour for spinous cells A sounder estimate will rest on additional experimental data on the values of  $T$  along with the mitotic indexes

#### COMMENT

The foregoing analysis points to a time sequence of differentiation in the strata of epidermis which may be described as follows Referring to the case in which it is assumed that the duration of mitosis,  $T$ , is  $\frac{1}{2}$  hour, table 3, a group of 10 basal cells will produce 10 new basal cells in 3,100 hours In the same period of time the adjacent 81 cells in the lower third of the spinous layer will produce 81 (3,100/1,050) or 24 new cells Similarly the 52 cells in the middle third of the spinous

layer will produce 52 (3,100/440) or 37 new cells, and the outer third of the spinous layer having 42 cells will produce 42 (3,100/1,330) or 98 new cells. Thus the entire spinous layer will generate a total of 24 plus 37 plus 10 = 71 new cells in 3,100 hours, while the 10 related basal cells will produce 10 new cells. The amplification in numbers of cells in the spinous layer is thus 71. The 71 new cells are produced at a rate of  $71/3,100$  or 0.023 cell per hour. If the number of cells in the granular layer is to remain constant at 42 cells, the 71 new cells will pass through the granular layer with a lifetime of  $42/0.023$  or 183 hours (7.6 days). Concerning the fate of the cells in the keratinized state, little can be said except that in a given case in which the mean life and the relative number of granular cells are accurately known, it is possible to estimate the rate of sloughing away if a count is made of the relative number of cornified cells present.

One feature which requires experimental study is the long lifetimes of basal and spinous cells. Sutton<sup>4</sup> estimated that the epidermis is renewed in a time of about 7 to 11 days. This estimate compares with the value of 7.6 days' and 7.9 days' lifetime of granular cells estimated in the foregoing paragraph. However, the basal and spinous cells are replaced in about 1,500 or 3,000 hours accordingly as one assumes for  $T$  a value of  $\frac{1}{4}$  or a value of  $\frac{1}{2}$  hour. Sutton's estimate is based on painting the epidermis (human) with 10 per cent silver nitrate solution and observing the rate of disappearance of the colored area. The calculated estimates of the granular lifetime suggest that the silver precipitate was associated with the cornified or granular layer rather than with the lower layers.<sup>4a</sup>

The foregoing analysis was carried out for the purpose of estimating, if possible, the relative rates of growth of basal and spinous cells. Tables 1 and 2 indicate that in an equal period of time the spinous cell will divide at least three times while the basal cell divides once. The ratio of 3 in the division rate of these two cells is comparable to the ratio of the growth rates of basal and epidermoid carcinoma as measured by Schrek.<sup>5</sup> The similarity between the data for normal scalp computed in the foregoing pages and Schrek's data for carcinoma may be fortuitous. Briefly stated, his data show that the median growth rate for basal cell carcinoma is 0.51 mm per month, while that for epidermoid carcinoma is 1.85 mm per month, the ratio being  $1.85/0.51 = 3.6$  (table 2).<sup>5</sup> The ratio of growth rates, however, does not give information about the absolute growth rate of the cancerous cells.

The sequence of differentiation considered in the numerical analysis of tables 1, 2 and 3 is based on the assumption that the countable

4 Sutton, R. L. Arch Dermat & Syph **37** 742, 1938

4a Current experiments show that the color due to silver nitrate painting is associated only with the cornified layer

5 Schrek, R. Arch Path **31** 422, 1941

nuclei represent cells and are derived from normal mitosis. No correction has been made for possible amitosis. Also, it is assumed that mitosis occurs uniformly at random in time and throughout the tissue. We assume that the "growth waves" suggested by Thuringer<sup>1b</sup> are averaged out in the average mitotic index which exists either over a long period of time or else over a large area of epidermis.

The computations indicate the importance of determining the actual mitotic indexes in the separate layers with the relative numbers of cells present in the several strata. I have taken a total mitotic index for the entire epidermal layer and partitioned it according to the relative numbers of cells and the relative incidences of mitoses as published by Thuringer. The single most important item lacking is the duration time of mitosis of each of the various types of cells of epidermis. This is a quantity of fundamental interest because it influences the mitotic count directly. For instance, it is desirable to know whether a cell such as the early spinous type, which multiplies rapidly, has a shorter duration of mitosis than the more slowly multiplying basal cell, and whether the duration time of mitosis can be correlated with multiplication rate. The computations presented herein serve to indicate the consequences of the fundamental assumption that all the cells of the stratified epidermis originate in the basal or "germinal" cells. The validity of the basic assumption that the basal layer is the generating layer cannot be established by the foregoing computations without additional experimental data.

#### SUMMARY

The relationship between the mitotic index, the duration time of mitosis and the cell number doubling time is applied to the growth of the strata of the epidermis of the scalp in which it is assumed that cells originate in the basal layer and by a process of differentiation become cornified cells. Thuringer's data on the mitotic activity in scalp are utilized to estimate the time sequence of the differentiation of cells in epidermis.

If the duration of mitosis is  $\frac{1}{2}$  hour, then the time sequence of differentiation is 3,100 hours as a basal cell, 2,900 hours as a spinous cell and 190 hours as a granular cell. The rate of division of spinous cells is three times that of the basal cells. This ratio of division rates is comparable to the ratio of growth rates of epidermoid and basal cell carcinoma as measured by Schrek.

# REGIONAL PLURICENTRIC HEMOLYMPHOLIPOBLASTOSIS

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SINCE Lobstein<sup>1</sup> first used the term "retroperitoneal sarcoma," in 1829, each case reported has added to the diversity of the histologic types of this tumor. Among the broad varieties which have been described, including simple epithelial cyst, composite dermoid teratoma, cystic adenoma, mesothelioma, leiomyoma, lymphangioma, lymphoma and neuroblastoma, those pertaining to the lipid system have attracted the greatest attention. Their structural appearance in many cases is that of a benign growth, yet they show a tendency to recur, thus behaving like a cancer. Among the 176 cases of retroperitoneal lipoblastoma analyzed by von Wahllendorf<sup>2</sup> there were 21 in which the growth became cancerous, and in 15 of the 115 in which the growth had been removed it recurred in a short time. This appears to be true also in the series of 30 cases with six deaths, six recurrences and three additional possible recurrences reported by Pemberton and Whitlock<sup>3</sup>. These authors demonstrated that in 14 per cent of their cases cancer was already present in the growth at the time of operation, a demonstration which justifies the opinion ventured by a number of authors that the tendency of these supposedly benign tumors to recur is due to the presence of sarcomatous elements which are often missed through incomplete histologic study. Another explanation that has been offered is that a number of recurrences of apparently benign fatty tumors represent small outgrowths overlooked at operation, so thought von Wahllendorf,<sup>2</sup> who, after the removal of a lipoma which weighed 7,000 Gm and during the final revision of the wound bed, discovered and removed fifteen additional small growths. Miller<sup>4</sup> also reported a case of multiple retroperitoneal lipomas, 4 of which, totaling 6,000 Gm in weight, were removed, whereas several others had to be left behind because of their closeness to vital structures.

Since by definition a primary retroperitoneal tumor arises from cellular elements included in the space comprised between the lumbar

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1 Lobstein, cited by Donnelly<sup>5</sup>

2 von Wahllendorf, A L. *Arch f klin Chir* **115** 751, 1921

3 Pemberton, J, and Whitlock, M E. *Surg, Gynec & Obst* **56** 110, 1933

4 Miller, J R. *Am J Obst.* **32** 652, 1936

and iliac regions, the peritoneum and the posterior parietal wall of the abdominal cavity (Donnelly<sup>5</sup>), cases in which there is an extension of the neoplastic process elsewhere in the abdominal cavity should not be considered in this category. Yet it must be more than incidental that there is frequent concomitance of retroperitoneal fatty tumors and fatty growths of a similar nature elsewhere in the abdominal cavity and even beyond it, in other parts of the body.

From the standpoint of localization, conditions diagnosed as lipoblastosis were divided by Goormaghtigh and colleagues<sup>6</sup> into two groups—the ones arising in the subcutaneous tissue and the ones arising in the internal cavities and deep organs. Among the conditions diagnosed as lipoblastosis of the internal cavities the authors distinguished fat tissue proliferation in a disorderly multicentric distribution affecting different parts of the body and fat tissue proliferation, either single or multiple, strictly limited to one region of the body (regional, localized or pluricentric lipoblastosis).

Since the retroperitoneal space is listed as the most frequent site both of the localized and of the pluricentric regional growths, there must be some truth in König's<sup>7</sup> suggestion of the occurrence of a diffuse anlage, extending from the space of Retzius to the kidneys, along the ureters.

In the cases of Hirsch and Wells<sup>8</sup> sites of fat tissue proliferation were the retroperitoneal space, the mesentery and other peritoneal folds. In Martland's<sup>9</sup> case the retroperitoneal space, the pelvis, the perirenal region, the mesentery of the small intestine and the gastrocolic omentum were involved by the proliferative process. In a case that I had the opportunity to study<sup>10</sup> fatty nodules were present along the course of the vessels of the left adrenal gland and in the mesentery of the ileum. The mesoappendix and the transverse mesocolon were infiltrated by similar nodular fat tissue growths and a large fatty mass almost completely obliterated the cul-de-sac of Douglas. Other sites of fat tissue new growths were the neck, the abdominal wall, the thigh, the popliteal space, the vertebral column and the thoracic cavity.

The ill defined limits among "lipopathies" and the frequent overlapping of morphologic aspects in the great variety of conditions in which overgrowth of fat tissue occurs explain the difficulties that are encountered in interpreting and classifying these extensive fat tissue

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5 Donnelly, B. A. Surg, Gynec & Obst **83** 705, 1946

6 Goormaghtigh, N., Vanderlinden, P., and de Puyssseleir, R. Cancer, Bruxelles **13** 3, 1936-1937

7 König, cited by Goormaghtigh and others<sup>6</sup>

8 Hirsch, E. F., and Wells, H. G. Am J M Sc **159** 356, 1920

9 Martland, H. Arch Path **5** 932, 1928

10 Tedeschi, C. G. Arch Path **42** 320, 1946



new growths This is clearly shown by the diversity of labels with which comparable observations have been deposited in the literature

There are on record cases of lipoblastosis which became locally progressive and exhibited wild potentialities of growth (Robertson<sup>11</sup>), and apparently similar cases in which gradual retrogression of the newly formed masses occurred (Mirolli<sup>12</sup>) Immature cells appearing in the new growth may lead to a false impression of a cancerous process (Jaffe<sup>13</sup>) and yet an entirely mature cell growth, arousing no untoward suspicion from the histologic standpoint, may later be the site of repeated recurrences (Tedeschi<sup>10</sup>) Ewing's<sup>14</sup> subdivided liposarcoma into two varieties, an adult fat cell type and an embryonal fat cell type, and the subdivision is generally accepted, but transformation from one cytologic type to another is not rare (Lang<sup>15</sup>), and there are, in addition, a number of cases in which the liposarcoma hardly fits into either of the two categories According to Jaffe<sup>13</sup> a sarcomatous change within the stroma of a fat tissue growth does not fulfil the qualifications of "liposarcoma" He also excluded from this category the mixed tumors in which fat tissue is associated with other types of tissue exhibiting evidence of cancerous change but in the growth of which the fat tissue per se seems to play a passive role He is also inclined to deny the sarcomatous nature of the lipoblastoma which, although composed of embryonic fat cells, shows little evidence of cellular anaplasia and in general absence of mitotic figures Along this line is my recent attempt<sup>10</sup> to segregate from the group of cases of sarcoma a number of well selected ones in the literature in which, although characterized by multiple recurrent fat tissue growths, the neoplasm fails to reveal, on histologic ground, any evidence of structural or cellular anarchy

That difficulties still exist in classifying is shown also by the case presented here, in which a systemic neoplastiform proliferation of the retroperitoneal fat and of practically all intra-abdominal fatty structures turned out, on microscopic study, to be characterized by a harmonic, and not at all anaplastic, proliferation of cells of the myeloid and of the lymphoid series and of young mesenchymal cells apparently lacking any lipid storage properties, the whole resulting in a tissue which was reminiscent of an embryonal preadipose fat tissue

The unusual features of the tumor, which hardly fits in any of the labeled categories of fat tissue overgrowths, are now described

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- 11 Robertson, H E J M Research **35** 131, 1916
  - 12 Mirolli, A Riforma med **44** 1624, 1928
  - 13 Jaffé, R H Arch Path **1** 381, 1926
  - 14 Ewing, J Neoplastic Diseases, ed 2, Philadelphia, W B Saunders Company, 1934
  - 15 Lang, W Arch f klin Chir **155** 349, 1929

## REPORT OF A CASE

L G, a 75 year old white woman, was admitted to the Framingham Union Hospital because of ascites of four months' duration. She could add little more to this history, and there were no clues as to the cause of the ascites. The chief finding on physical examination was a large abdomen so distended with fluid that no organs or masses could be palpated. Rectal examination revealed nothing of note, and because of the ascites pelvic examination was unsatisfactory. A peritoneoscopic examination was made, but only a small amount of ascitic fluid could be obtained and nothing was visualized.

Approximately one hour after the operation the patient went into severe shock and became pulseless, pale and sweaty, the systolic pressure dropped down to 60, and marked tenderness developed on the right side of the abdomen where the peritoneoscopic procedure had been performed. Despite the administration of oxygen, of cardiokinetic stimulants and of a promptly delivered blood trans-



Fig 1—Specimen from the greater omentum (detail in actual size) showing marked lobulation resulting in irregularity of the surfaces

fusion, the patient did not come out of shock and died three and one-half hours after the operation.

*Summary of the Autopsy Record*—When the usual Y-shaped incision was made, a large hematoma extending from the right costal margin to the iliac crest was found in the thickness of the right abdominal wall. The blood, in an estimated amount of 500 cc, was partly clotted and partly still in a fluid state. More blood clots were found free in the abdominal cavity, floating in a chylous exudate of an estimated amount of 2,000 cc.

The greater omentum covered most of the intestines and was unusually thickened throughout, from 3 to 7 cm in the different areas. It was firm in consistency and markedly lobulated in appearance, although no actual nodular masses could be felt. Both externally and on the cut section the color varied from yellowish gray to yellowish pink, with fine bands of glistening pale gray tissue crossing one another in network fashion (fig 1).

When the omentum was pushed aside, an identical appearance was shown by the mesentery of the ileum, by the epiploic appendices, by the mesoappendix and by the mesocolon throughout, all of which were unusually thickened (figs 2 and 3) The retroperitoneal fat also was greatly increased in amount and appeared as a resilient mass which indistinctly merged into sausage-shaped fatty

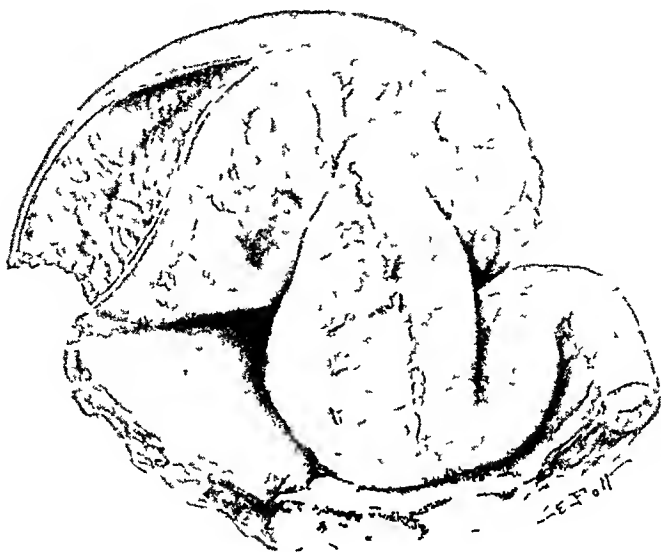


Fig 2—Specimen from a representative segment of the small intestine, including the thickened mesentery, showing the stiff appearance of the loops, which are coated throughout with a layer of yellowish gray lardaceous tissue



Fig 3—Specimen from a portion of the sigmoid flexure enveloped by the overgrown fatty tissue, which merges into the thickened epiploic appendices

masses which surrounded the vertebral column, following the course of the large blood vessels up and beyond the iliac bifurcation

The cul-de-sac of Douglas was almost completely obliterated by a yellowish gray, pinkish tissue which in all respects resembled the omental tissue and the fat tissue in the other abdominal localizations From the cul-de-sac the fatty

mass had extended toward the broad ligaments, which appeared as two thick, fringed aprons completely enveloping the uterus, the tubes and the ovaries

Both the large and the small bowel had a stiff appearance and were covered throughout with a thick layer of yellowish gray lardaceous tissue which smoothly merged into the enormously thickened epiploic appendixes. On coronal section it was clear that the fatty infiltration of the intestinal wall did not extend beyond the serosa, both mucosa and muscular coats appearing grossly normal. The fatty capsule of the kidneys was also thickened, averaging from 3 to 5 cm, the usual soft yellow-colored tissue was replaced at many points by a firmer and paler tissue which indistinctly blended into the normal fat tissue. The mesenteric and periaortic lymph nodes could hardly be detected in the overgrown fat tissue and did not appear to be unusual in size, shape or any other characteristic.

A chylous fluid, identical in character with that found in the abdominal cavity, was present in both pleural spaces, 400 cc in the right and 300 cc in the

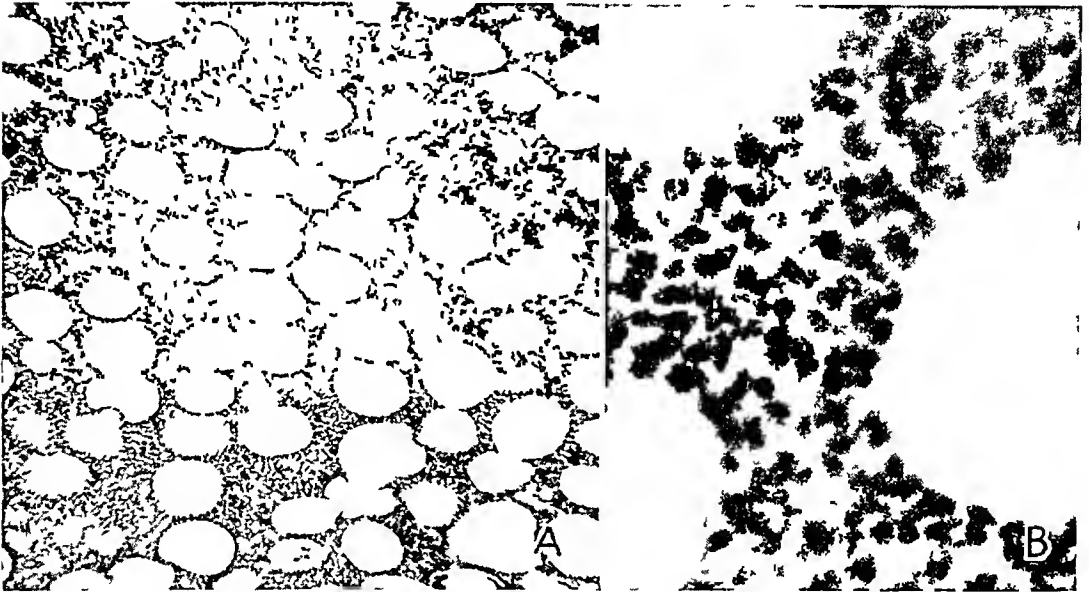


Fig 4—*A*, low power view of the greater omentum showing fatty spaces separated widely, one from the other, by a harmonious infiltration of cells. Regardless of the localization, this was invariably the structure displayed by the intra-abdominal fatty masses. Photomicrograph, ocular 5, objective 10, Zeiss.

*B*, area from the same field at higher magnification showing large lymphocytoid cells, interpreted as histiocytes, and darker smaller cells with characteristics of lymphocytes. Photomicrograph, ocular 10, objective 40, Zeiss.

left. On investigating the cause of this condition, attention was brought to the thoracic duct. Its lower third, over a tract 3 cm in length, was found to be markedly dilated, up to the circumference of a pencil. On sectioning, this dilated portion was seen to be located above a yellowish gray mass the size of a pea, friable, firmly adherent to the duct's walls and occluding the lumen almost completely.

Additional findings were hypertrophy and dilatation of the left ventricle of the heart, accompanied with mild generalized myocardial fibrosis, diffuse arteriosclerosis, most severely affecting the renal vessels and resulting in bilateral nephrosclerosis, congestion of the lungs, the liver and the spleen.

None of the fat deposits in the body appeared to be unusual, and the subcutaneous fat, which at the level of the abdominal wall averaged 15 cm in thickness, failed to reveal any deviation from the normal

*Microscopic Observations*—Regardless of the localization of the intra-abdominal fatty masses their structural characteristics were invariably the same. Under low power magnification the striking pattern was that of fatty spaces, either sparse or in small groups, separated from one another by a harmonious and diffuse infiltration of cells (fig 4), the whole resulting in a tissue which was reminiscent of hyperplastic bone marrow. On closer examination the cells taking part in the infiltration were seen to fall into one or another of the following categories: (a) the most common cell type basically resembled a lymphocyte (fig 5). It differed from the usual lymphocyte, however, because of the more coarsely granular appearance of the nuclear chromatin, the less pronounced cytoplasmic basophilia and the cellular outlines that were often polyhedral instead

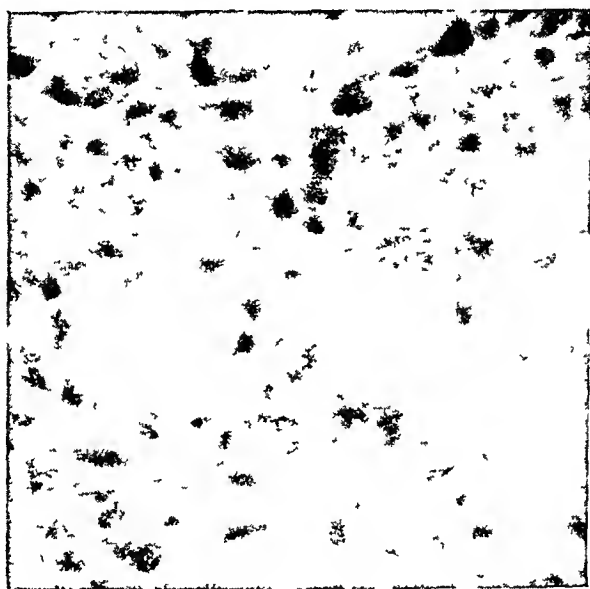


Fig 5—Area from another section of the omentum including immature cells of the myeloid series. Photomicrograph, ocular 5, objective 40, Zeiss

of round or oval. A few of the nuclei were small, but the majority were large, occupying from one half to two thirds of the cellular diameter. An occasional nucleus contained one or two nucleoli or a single large nucleolar structure almost as large as the entire nucleus. The cytoplasm was narrow, dense and basophilic.

(b) Irregularly intermixed with these cells, whose characteristics closely resembled the lymphocytoid cells (Maximow<sup>16</sup>, Tedeschi<sup>17</sup>) of the connective tissue (generally interpreted as histiocytes), were typical lymphocytes, and what seemed to be transitional patterns between the two cell types could be recognized here and there. In some areas the lymphocytoid cells prevailed, whereas in some other areas the lymphocytes were predominant.

16 Maximow, B. Textbook of Histology, Philadelphia, W. B. Saunders Company, 1934.

17 Tedeschi, C. G. Arch. ital. di sc. med. 13: 257, 1932.

(c) In the midst of these cells, lymphocytoid in character, a few spindle cells, thickly interlaced, and large, irregularly stellate cells with branching cytoplasmic processes, often fused together in syncytial masses, could be seen, but they were far from being numerous or otherwise conspicuous

(d) Among the non-fat-bearing cells immature hematic cells, including myelocytes and nucleated red cells, were also present, either sparse or coalesced into small nodular formations which assumed the appearance of foci of extramedullary hemopoiesis (fig 3) In the areas where the lymphocytoid cells were exclusive no intercellular substance could be recognized, where the fibroblasts and the undifferentiated branching cells were present, a few delicate interlaced reticulum fibers could be seen and in their meshes there was an amorphous eosinophilic material which could not be stained with mucicarmine No mitotic figures or any other pattern which might be suggestive of an anaplastic cellular growth were ever seen Staining for fat droplets revealed none in the cells infiltrating between the fat spaces in spite of occasional cytoplasmic vacuoles seen in the sections stained with hematoxylin and eosin

Blood capillaries could not be seen in the most cellular areas, where the cells were less abundant, capillaries could be recognized and were found not to be unusual either in number or in character The fat cells showed evidence of simple atrophy in the absence of retrogressive change or of any attempt at cellular proliferation

Without variations, this was the appearance of the outgrowing fat tissue throughout the abdominal cavity Where the fatty masses lay close to the abdominal organs, the cellular infiltrate that had so widely replaced the fat tissue stopped abruptly and in no instance was seen to cross over the fat itself and to penetrate into the adjacent structure This appeared to be true in broad sections, including the perirenal fatty capsule and the kidney proper, which was found to be immune to any cellular infiltration, and in coronal sections both from the small and the large bowel, in which the cellular infiltration was seen to stop abruptly at the limits between the fatty serosa and the subjacent, perfectly normal muscular coat An identical behavior was displayed by the sections from the uterus, the fallopian tubes, the ovaries and the adrenal glands, which, in spite of being enveloped by the outgrowing fat, were found to be free from cellular invasion

That the retroperitoneal fat tissue and the other intra-abdominal fatty structures were the only ones involved by the process was shown by the absence of any similar change in any of the remaining organs of the body Particular attention was given to the spleen, to the abdominal and extra-abdominal lymph nodes and to the intestinal lymph follicles None of these lymphoid structures were found to be unusual except for a good deal of fatty replacement of the lymph nodes in the mesentery Smears of sternal marrow failed to reveal any deviation from the normal Blocks of subcutaneous fat, of mediastinal fat and of the fat tissue of the axillary fossa did not show any increase in cellularity or any other pattern which might suggest even an early stage of a process comparable to that detected in the abdominal fat

A structure identical with that displayed by the abdominal fat was shown, however, by the mass occluding the thoracic duct, the lumen of which was seen to be occupied by a few fat spaces widely infiltrated by cells which in all respects resembled the cells infiltrating the fatty structures of the abdominal cavity At the level of the occlusion the walls of the thoracic duct were thin but otherwise not remarkable except for patchy areas showing sloughing off of the lining endothelium

## COMMENT

A systemic outgrowth of the fat tissue of the retroperitoneal space and of practically all intra-abdominal fatty structures, including the greater omentum, the mesentery of the ileum, the epiploic appendixes, the entire mesocolon, the mesoappendix, the broad ligaments, the cul-de-sac of Douglas and the fatty capsule of the kidneys, was the unusual characteristic of the case here reported, which from the standpoint of a gross description falls into the category of the regional pluricentric lipoblastosis of the classification given by Goormaghtigh, Vandeilinden and de Puyssleyn<sup>6</sup>

When one tries to explain the nature of the process, the first question is whether the outgrowing fatty masses were the exponent of a widely spreading lipoblastic sarcoma that started in one or another fatty area within the abdominal cavity, or whether they were a manifestation of a systemic process which independently had involved all the abdominal fatty structures. If one considers the outgrowing fatty masses to have been independent, the second question is whether they were cancerous in the strict meaning of the term, or whether they were the expression of a proliferative process of a different nature.

The outgrowing fatty masses differed a good deal from the normal fat in color and consistency, yet they lacked the nodular distribution which is generally met with in the usual tumor implants, furthermore, no organ or structure could be incriminated as the primary source of the neoplastic process, only the presence in the thoracic duct of what obviously evidenced a fat cell invasion of the system of the lymph channels inclined the observer toward the conception of a cancerous process.

Regardless of the localization, in none of the numerous microscopic sections of the various fatty masses was evidence found of mitotic figures or of cellular anarchy, the structure being invariably that of an interlacing of lymphocytoid cells, of embryonal connective tissue cells and of immature hematic cells of all series infiltrating the fatty spaces.

These being the main features of the process, it is apparent that the case does not fit in any of the categories of lipid tissue new growths, there was nothing to suggest an adult fat cell type of liposarcoma, characterized by rounded or polygonal granular cells simulating closely those found in chronic inflammation of fat tissue, the embryonal fat cell type of liposarcoma could also be excluded in the absence of patterns suggesting arrest in the differentiating of embryonal mesenchymal cells into fat cells, with exhibition of either myxomatous or foamlike properties.

A sarcomatous change within the stroma of the fat tissue or a propagation in the fat tissue stroma of a cancerous new growth started

elsewhere, although not fulfilling the qualification of liposarcoma (Jaffe <sup>18</sup>), has also to be given some thought but, as already mentioned, no mitotic figures or any other evidence of cellular anaplasia could be noticed among the proliferated cells and no structure in the body, including the lymphatic and hemopoietic systems, displayed patterns which could even be doubtfully of a neoplastic process

Turning to other pathologic conditions—another possibility which must be considered is that the systemic proliferation of cells within the abdominal fat might have been the exponent of an inflammatory response to some sort of injurious agent

Reactive phenomena in the fat tissue do not differ from those in other organs, the histologic details, however, are peculiar to the special nature of the tissue involved. An inflammatory response in the fat tissue may fall in one or another of the following three types: (a) a nonspecific response, ultimately leading to a process of fibrosis, (b) a tuberculoid type of process, mainly characterized by a proliferation of epithelioid cells and of fixed connective tissue cells and (c) a phagocytic response. The distinction must then be made clear between those conditions in which the inflammatory reaction is elicited by the propagation in the fat tissue of an injuring agent that primarily has affected an adjacent structure and those in which the adipose tissue is primarily affected. The terms "primary" and "secondary panniculitis" may be used to indicate these different conditions.

As none of the organs within the abdominal cavity or of the organs elsewhere in the body showed any evidence of inflammatory processes, it is apparent that if the abdominal fatty changes in this case were to be considered of an inflammatory nature they would necessarily fall under the category of the so-called primary panniculitis.

The first mention of the possibility that the fat tissue may be the site of a primary and independent inflammatory process is attributed to Pfeiffer <sup>18</sup> (1892). Many years later, in 1916, Gilchrist and Ketron <sup>19</sup> added new evidence in favor of this conception but it was only in 1925 that, owing to Weber,<sup>20</sup> the process was recognized as a distinct clinical and pathologic entity. Since the process was mainly characterized by recurrent crops of subcutaneous nodules occurring during febrile periods, and the underlying pathologic finding consisted mainly of fat atrophy and inflammation in the absence of any other concurrent detectable lesion, he termed the condition "relapsing, non-suppurative nodular panniculitis."

18 Pfeiffer, V. *Deutsches Arch f klin Med* **50** 438, 1892

19 Gilchrist, T. C., and Ketron, L. W. *Bull Johns Hopkins Hosp* **27** 291, 1916

20 Weber, F. P. *Brit J Dermat* **37** 301, 1925



The description of Christian<sup>21</sup> may well be taken as representative of the underlying pathologic process, characterized by edema and necrosis of the entire fat lobule, by the infiltration between the fat cells of lymphoid cells, plasma cells, young connective tissue cells, endothelial cells phagocytic for fat droplets, a few polymorphonuclear leukocytes and rare foreign body giant cells and by occasional vascular changes of the periarteritic type. To these characteristics Bailey<sup>22</sup> added the almost consistent integrity of the interlobular connective tissue septums—hence his impression that the essential change resulted in the appearance of lipophagic cells (variously interpreted as histiocytes, polyblasts or granuloma cells) around the smaller blood vessels within the fat lobule.

What at first was thought to be a localized disease of the subcutaneous fat tissue was found to have more extensive roots in the body when Miller and Kritzler<sup>23</sup> published the first fatal case of panniculitis. Besides the subcutaneous lesions, fatty changes and focal necrosis of the liver, hydropic degeneration of the adrenal cortex, phagocytosis of red cells in members of the reticuloendothelial system and fat embolism of the lungs were displayed at postmortem studies. Changes similar to those in the subcutaneous fat were found by Spain and Foley<sup>24</sup> in the mesenteric, in the mediastinal and in the omental fat, in another fatal case observed by Mostofi and Engleman<sup>25</sup> the epicardial, peripancreatic, periadrenal, perirenal and mesenteric fat tissues were found to be involved by the process.

On perusing the literature one finds, however, little agreement as to the processes that should be considered under the heading of Weber-Christian disease. As changes similar to those described under this heading were seen to occur following a mechanical or a chemical damage of the fat tissue in the absence of any significant clinical symptom, some investigators have been inclined to consider "non-suppurative panniculitis" not a disease entity but a syndrome likely to be brought about by a number of injurious agents. Considering that a nonsuppurative type of panniculitis is often encountered but that only in a limited number of instances is it accompanied by the cortege of symptoms characterizing Weber-Christian disease, one feels it is reasonable to accept the suggestion of Keil<sup>26</sup> that the term "nonsuppurative panniculitis" be used in the general descriptive sense encompassing all pathologic states

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21 Christian, H. A. *Arch Int Med* **42** 338, 1928

22 Bailey, R. J. *J A M A* **109** 1419, 1937

23 Miller, J. L., and Kritzler, R. A. *Arch Dermat & Syph* **47** 82, 1943

24 Spain, D. M., and Foley, J. M. *Am J Path* **20** 783, 1944

25 Mostofi, F. K., and Engleman, E. *Arch Path* **43** 417, 1947

26 Keil, H. *Brit J Dermat* **47** 512, 1935

characterized by inflammatory response of the fat tissue—mainly on the part of fixed connective tissue cells—and to reserve the term “relapsing, febrile, nodular, nonsuppurative panniculitis” as synonymous with Weber-Christian disease

This being the situation, Weber-Christian disease is out of question in the case under consideration, as there were none of the symptoms, and the distributions of the lesions were not those encountered in that condition. As for a nonsuppurative type of panniculitis, the cellular reaction usually found in such a condition compares to a considerable extent with the reaction encountered in the case under discussion, but in the latter there was none of the edema, necrosis and angitis that almost invariably accompany the tissual change of a panniculitis

When the gamut of cells characterizing the fat tissue overgrowths of the case under study is compared with that entering into the composition of the embryonal preadipose tissue, it is apparent that the two have close resemblances. A peculiar “open-meshed” tissue, resulting from the interlacing of fine connective tissue fibers and of loosely arranged cells with two or more long, coarse processes, was seen by Bell<sup>27</sup> to precede the formation of adipose tissue. As fat began to be deposited, the branched preadipose cells became rounded and their processes absorbed. In agreement with these early observations is Ferrata’s<sup>28</sup> inclusion of the fat cell among the cells of the hemohistioblastic system and Wasserman’s<sup>29</sup> conception of an origin of fat cells from perivascular mesenchymal cells related to the reticulum. He depicts the formation of the “primitive fat tissue” as being preceded by the formation of a preadipose tissue consisting of blood capillaries and of perivascular histiocytic cells anastomosing one with another by means of branching processes. The interstices of the resulting network are later invaded by argentaffin fibers and by fenestrated plasmatic membranes, in which fat becomes deposited. In between the evolving adipose cells he recognized hematic cells of different types (megaloocytes, myeloblasts, plasmatic cells), which he thought had originated also from the pluripotent periadventitial mesenchymal cells. This has been shown also by Cioni,<sup>30</sup> who described the formation of hematic cells in the fat tissue as being preceded by a differentiating of the histiocytes into polyblastic cells.

The frequent occurrence of foci of hemopoiesis in the fat tissue of the adult under a variety of pathologic states (aplastic anemia, leukemia)

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27 Bell, E. T. *Am J Anat* 9 412, 1909

28 Ferrata, A. *Le emopatie casa*, Ed. Vallardi, 1914

29 Wasserman, J. A. *Ztschr f Zellforsch u mikr Anat* 3 235, 1926

30 Cioni, C. *Arch de Vecchi* 1 343, 1939

is generally interpreted as a revival of this embryonal condition. Foci of hemopoietic tissue in the retroperitoneal fat have been shown by Petri,<sup>31</sup> most frequently in persons dying of acute infections. Immature blood cells within a fat tissue tumor have been described by Blaisdell,<sup>32</sup> and Babes<sup>33</sup> mentioned that normoblasts, megakaryocytes and plasma cells were present in a recurrent mesenteric lipoma. In describing a cancerous, nonlipid retroperitoneal tumor, Warren<sup>34</sup> stressed the presence of foci of immature hematic cells, which he interpreted as originating from embryonal mesenchymal cells of the retroperitoneal fat tissue. I offered the same interpretation in a discussion<sup>10</sup> of large foci of immature hematic cells in a case of multiple independent fat tissue growths. Cases of adrenal myelolipoma are on record. Collins<sup>35</sup> divided them into two categories, in one the tumor is yellowish orange and on microscopic examination is found to have a predominance of adipose tissue with minimal myeloid elements and a large proportion of erythroblastic cells, in the second the tumor is dark red grossly and microscopically shows a predominance of myeloid elements and minimal erythroblastic proliferation.

As for the lymphocytoid cells, so largely represented in the case under study, a number of students have stressed the significance of a cell lymphocytoid in character, resembling to a considerable extent a plasma cell in the embryonal connective tissue in which fat droplets later become deposited (Waldeyer<sup>36</sup>), Bobritzky<sup>37</sup>, Poljakoff<sup>38</sup>). Geschickter,<sup>39</sup> in reviewing a large series of lipid tumors, stressed the presence in cancerous growths of a cell type resembling a "plasma cell or fetal cartilage" and pointed out the possibility that it might represent the forerunner of the larger foam cell. According to Jordan,<sup>40</sup> it is from this lymphocytoid cell that, through an intermediary hemocytoblastic stage, hematic cells are being formed in the fat tissue. Distinct transitional patterns between these lymphocytoid cells and cells with characteristics of histiocytes on one side and with features of lipoblasts on the other were recognized by me in the fatty portion of a renal

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31 Petri, E. Virchows Arch f path Anat **258** 37, 1925

32 Blaisdell, J L. Arch Path **16** 643, 1933

33 Babes, A. Bull Assoc franç, p l'étude du cancer **18** 334, 1929

34 Warren, S. Am J Path **4** 51, 1928

35 Collins, D C. Am J Path **8** 97, 1932

36 Waldeyer. Arch f mikr Anat **11** 176, 1875

37 Bobritzky. Centralbl f d med Wissensch **23** 753, 1885

38 Poljakoff. Arch f mikr Anat **32** 122, 1888

39 Geschickter, C F. Am J Cancer **21** 617, 1934

40 Jordan, H E. Anat Rec **59** 461, 1934

Wilms tumor,<sup>41</sup> and I saw comparable cellular forms in early developmental stages of an experimentally produced embryonal cell liposarcoma<sup>42</sup>

The cyclic alternations between lymphoid and adipose tissue have been commented on by a number of investigators. In embryonal development the formation of lymphoid tissue may precede or be coincident with the formation of the primitive fat tissue. In old age the lymphoid structures undergo atrophy and are progressively replaced by fat tissue, this explains the not at all rare occurrence of lymph nodes that come to be represented by a lobule of fat tissue surrounded by a well defined capsule and containing blood, lymph vessels and fibrous septums. The occurrence of large foci of cells lymphocytoid in character in the fat deposits of splenectomized animals (Tizzoni and Fileti<sup>43</sup>, Wino-gradow<sup>43</sup>, Tedeschi and Santoro<sup>44</sup>) may also be explained as a compensatory revival of embryonal characteristics of growth. In describing the sequence of events leading to the formation of these lymphoid areas, Warthin<sup>43a</sup> pointed out, first, the dilatation of blood capillaries, then the removal of the fat from the cells along the dilated capillaries, the conversion of these cells into reticular cells and finally the development of the lymphoid tissue into the meshes of the reticulum. A finding similar to that presented by the splenectomized animal is frequently found in man under a variety of conditions. In carcinoma of the breast and even in lactation an increase in size and perhaps in number of the axillary glands is a common occurrence and is generally interpreted as due to new formation of lymphoid tissue in the axillary fat. The same is true of the frequent hyperplasia of lymphoid tissue in the omentum and mesentery in a variety of intestinal processes and even in the absence of any plausible explanation. In all these cases as the lymphoid cells extend in cords from the lymph nodes into the fat tissue septums and the outlines of the lymph nodes themselves become indefinite, it is difficult to say whether the lymphoid cells arose in situ from pluripotent mesenchymal cells of the fat tissue stroma or branched from the bulk of the lymph follicles into the surrounding fat tissue.

From the foregoing considerations one might feel encouraged to regard the great variety of cellular forms encountered in the abdominal fatty masses as an expression of the multiple developmental potentialities

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41 Tedeschi, C. G., Holtham, W. H., and Minor, C. Mixed Tumors of the Kidney, *J. Urol.*, to be published.

42 Tedeschi, C. G. Experimental Embryonal Cell Liposarcoma, *Arch. Path.* to be published.

43 Cited by Warthin, A. S. *Proc. Path. Soc. Philadelphia* 6:12, 1903.

43a Warthin<sup>43</sup>

44 Tedeschi, C. G., and Santoro, A. M. *Arch. di fisiopat.* 2:146, 1934.

of the undifferentiated mesenchymal cells of the fat lobule which under a stimulus, the nature of which is not clear, exhibited a revival of their embryonal properties

If this is true, the condition might properly be labeled under the term "hemolympholipoblastosis, regional, pluricentric," to indicate (*a*) the limitation of the condition to the fat tissue of a determined region of the body (in this specific case the abdominal cavity), (*b*) its systemic multicentric localization, and (*c*) the predominance of elements of the myeloid and lymphoid series in the cellular growth

It is possible that a number of observations corresponding to the characteristics outlined in the foregoing paragraph have been previously deposited in the literature. Most likely they are to be found among the reports of cases of lymphosarcoma or of lipoblastic sarcoma. Against such interpretations in the case here described are, however, the lack of evidence of cellular anarchy, of mitotic figures and of any other cellular or structural pattern suggesting a cancerous growth either autochthonous or originating elsewhere in the body, the absence of lipid storage properties of the proliferated cells, and the passive role played by the tissual lipid cells

The start of the process in this case and the signs and symptoms that accompanied its development are not known, nothing can be said, therefore, on the course, the duration and the outcome of the condition. To judge from the cytologic characteristics of the process, its aggressive properties seem to be limited, yet the blocking of the thoracic duct by cells carried into the lymph stream suggests that one should exercise a good deal of prudence on this point. This occurrence, that certainly had much to do in precipitating the course of events, might have been brought about by unusually favorable interrelations of growth between the lymph channels and the proliferated cells, but one cannot even categorically rule out an intrinsic aggressive potentiality of the proliferated cells which in other cases might show itself with more pronounced manifestations

#### SUMMARY

The case is reported of a 75 year old woman with a history of ascites of four months' duration who died under shock following peritoneoscopy. The outstanding finding at postmortem examination was a systemic outgrowth of the intra-abdominal fat, including that of the retroperitoneal space, the greater omentum, the mesentery of the ileum, the epiploic appendixes, the entire mesocolon, the mesoappendix, the broad ligaments, the cul-de-sac of Douglas and the fatty capsule of the kidneys. In all localizations the fat tissue outgrowth was found to be characterized by a harmonic, and not at all anaplastic, proliferation of

young mesenchymal cells, apparently lacking any lipid storage properties, and of cells of the myeloid and lymphoid series, the whole resulting in a tissue which was reminiscent of an embryonal preadipose tissue

The process is labeled with the term "hemolympholipoblastosis, regional, pluricentric," to indicate the limitation of the condition to the fat tissue of a determined region of the body (in this specific case the abdominal cavity), its systemic multicentric localization and the predominance of elements of the myeloid and lymphoid series in the cellular growth

In trying to interpret the genesis of the process, the great variety of cellular forms is regarded as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cells of the fat lobule, which under a stimulus, the nature of which is not clear, exhibited a revival of their embryonal properties

To judge from the cytologic characteristics of growth and the complete absence of mitotic figures and of any other evidence of cellular or structural anarchy, the aggressive properties of the process seem to be limited, however, the blocking of the thoracic duct by a growth of cells identical in character with those in the abdominal fat suggests other possibilities

# INTUSSUSCEPTION, FECAL IMPACTION AND VOLVULUS PRODUCED BY INJECTION OF PILOCARPINE

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**I**NTUSSUSCEPTION is not an uncommon disease, and its clinical and pathologic aspects have been described by many authors. In adults and older children the genesis of the lesion often can be explained by some intrinsic disease of the intestine, such as a tumor, or by the presence of Meckel's diverticulum. Such cases, however, in which an anatomic lesion serves to explain the origin of the intussusception, actually are in the minority, and most instances of the disease are without obvious cause. In almost three fourths of the reported cases the patients have been children of less than 2 years of age, and in these the cause of the lesion was usually obscure.<sup>1</sup>

The disease is not confined to man. It has been observed in a wide variety of animals, including horses, cows, hogs, sheep and fowl,<sup>2</sup> dogs,<sup>3</sup> skunks,<sup>1</sup> mice<sup>4</sup> and monkeys.<sup>5</sup>

There have been relatively few experimental studies of intussusception. In 1884 Nothnagel<sup>6</sup> produced the lesion by directly stimulating the intestine with a faradic current, and in 1910 Propping<sup>7</sup> was able to

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1 (a) Wangenstein, O. H. *Intestinal Obstructions*, ed 2, Springfield, Ill., Charles C. Thomas, Publisher, 1942, chap 21, pp 396-420. (b) Tumen, H. J., in Bockus, H. L. *Gastro-Enterology*, Philadelphia, W. B. Saunders Company, 1944, vol 2, chap 55.

2 Hutyla, F., and Marek, J. *Special Pathology and Therapeutics of the Diseases of Domestic Animals*, ed 3, Chicago, Alexander Eger, vol 2, pp 299-307, 1926.

3 Malcom, J. D. *Brit M J* 2:809, 1923. Graham, J. Personal communication to authors. Hutyla and Marek.<sup>2</sup>

4 Personal observation by the authors.

5 (a) Watts, J. W., and Fulton, J. F. *New England J Med* 210:883, 1934. (b) Fulton, J. F., Kinnard, M. A., and Watts, J. W. *Proc Am Physiol Soc* 1934, in *Am J Physiol* 109:37, 1934.

6 Nothnagel, cited by Wangenstein.<sup>1</sup>

7 Propping. *Mitt a d Grenzgeb d Med u Chir* 21:536, 1910.

induce the disease in rabbits by injecting physostigmine sulfate directly into the lumen of the intestine. The report<sup>8</sup> of intussusception in a cat following administration of ice water is an isolated observation. Perhaps the most extensive work is that of Watts and Fulton,<sup>5a</sup> who observed spontaneous fatal intussusception in 3 monkeys in which the region of the premotor area of the cortex of each cerebral hemisphere had been removed. Subsequent experiments showed that faradic stimulation of the premotor area gave rise to active peristaltic movements of the intestines, and in two experiments continuous stimulation produced multiple intussusception. In 3 instances intermittent stimuli also produced intussusception. The authors were unable to produce the lesions after section of the vagi. In a later study they and Kinnard<sup>5b</sup> observed peristaltic action in the intestine of a chimpanzee following use of similar stimuli.

Intussusception also has been produced by mechanical invagination of the intestine of the cat,<sup>9</sup> and Ravitch and McCune<sup>10</sup> recently produced lesions in dogs by a similar method and were able to study methods of reduction and pathologic features of intussusception.

#### MATERIALS

The guinea pigs were obtained from commercial breeders. One group had been in our laboratory for over six months, these averaged 620 Gm in weight and were called adult animals. The others were small, immature, rapidly growing guinea pigs and averaged 305 Gm, this group was termed young animals.

Pilocarpine hydrochloride was used, and was administered either in a saline solution or in an oily medium. The latter was a beeswax-liquid petrolatum mixture prepared by adding 5 Gm of white beeswax to 95 Gm of liquid petrolatum and mixing in a Warring blender. The pilocarpine hydrochloride was ground to powder in a dry porcelain mortar, and the oily mixture was then added and mixed in the same mortar. The product used was 2 per cent pilocarpine hydrochloride in the oily medium, this suspension was quite stable, settled out slowly and could be injected with a no. 20 needle. It was not sterilized before injection and was kept from contact with water.

#### EXPERIMENTS

*Determination of the Effects of Pilocarpine Hydrochloride in Saline Solution and Pilocarpine Hydrochloride in an Oily Medium on Fed Young Guinea Pigs, Methods and Results*—Young guinea pigs were kept in the laboratory for a period of observation lasting over a week, during which they had constant access to grain and were given lettuce or similar greens each morning. On the morning of the injection of pilocarpine hydrochloride the animals were weighed after they had eaten their greens. In the afternoon, usually three or four hours after the animals' feeding, the substances to be tested were injected subcutaneously.

In the first experiment, one half of each group received the test drug, the other the bland substances, subsequently all animals were first given an injection of bland

8 Britton, S. W. *Virginia M. Monthly* **56** 515, 1929.

9 Power, D. *Brit. M. J.* **1** 381, 453 and 514, 1897.

10 Ravitch, M. M., and McCune, R. M. *Bull. Johns Hopkins Hosp.* **82** 550 1948.



substances The animals given the drug exhibited erection of hair, salivation, lacrimation, diarrhea and dehydration, frequently leading to prostration and occasionally death

In the guinea pigs stimulated by pilocarpine a total of 4 intussusceptions, 2 volvuli, and 4 fecal impactions were observed (table 1) The anatomic changes are discussed subsequently They were considered sufficient to account for the deaths of the animals No lesions were observed in the animals receiving only the saline solution or the oily medium

This technic produced relatively few lesions, but their statistical significance was great enough to encourage further investigation (chi, or deviation, divided by the standard deviation being 4.2 for intussusception, 3.5 for volvulus and 4.2 for fecal impaction as calculated by the fourfold method<sup>11</sup>)

Although the differences between the groups receiving pilocarpine in saline solution and those receiving the drug in an oily medium were not great, it was decided to use the latter in subsequent experiments, because of its greater efficacy, and because the signs of toxicity persisted for a much longer period (often ten or fifteen hours) when the drug was given in the oily medium

TABLE 1—*Effect of Subcutaneous Injection of Pilocarpine Hydrochloride on Young Guinea Pigs\**

| Substance Injected             | Dose,<br>Cc per Kg | Animals            |                   |                   | Total<br>Number<br>of<br>Injec-<br>tions† | Deaths Due to |                           |               |                         |
|--------------------------------|--------------------|--------------------|-------------------|-------------------|---|---------------|---------------------------|---------------|-------------------------|
|                                |                    | 1st In-<br>jection | 2d In-<br>jection | 3d In-<br>jection |   | Tox-<br>icity | Intus-<br>suscep-<br>tion | Vol-<br>vulus | Fecal<br>Impac-<br>tion |
| Saline solution                | 1 7 2 4            | 45                 | 43                | 33                | 121                                       | 0             | 0                         | 0             | 0                       |
| 0.5% pilocarpine hydrochloride |                    |                    |                   |                   |   |               |                           |               |                         |
| in saline solution             | 1 7 2 4            | 48                 | 45                | 8                 | 101                                       | 3             | 1                         | 0             | 2                       |
| Oily medium §                  | 1 5 2 5            | 82                 |                   |                   | 82  | 0             | 0                         | 0             | 0                       |
| 2% pilocarpine in oily medium  | 1 5 2 5            | 89                 |                   |                   | 89  | 19            | 3                         | 2             | 2                       |

\* The animals were fed mixed grains and lettuce greens each morning The injections were performed in the afternoon

† These injections were given at intervals of from three to four days

§ This was 5 per cent beeswax in liquid petrolatum

One group of survivors of the experiments, shown in table 1, originally numbering 31 animals, all of which had been previously given an injection of pilocarpine hydrochloride in the oily medium, were given weekly injections of 16 cc per kilogram of this suspension Three intussusceptions were observed after the second injection of this substance, one after the third and one after the seventh, when only 8 animals remained and the dose had been increased to 24 cc per kilogram It was concluded that failure of an animal to show intussusception on the first injection did not preclude the development of such lesions after subsequent injections

*Determination of the Extent to Which Age and Food Intake Were Related to the Development of Intussusception*—Two groups of animals were used, one of the groups, called adult, had been in the laboratory for over six months and averaged 620 Gm in weight, the second group of animals, called young, was made up of recently purchased small animals, averaging only 305 Gm They grew rapidly during the experiments All animals were observed for one week before the experiment while being fed the laboratory diet of mixed grains and greens

On the morning of the day before the experiment the animals were fed grain and greens as usual, then weighed This weight was used to calculate the dose of

11 Simmonds, J S, and Gentzkow, C J Laboratory Methods of the United States Army, ed 5, Philadelphia, Lea & Febiger, 1944, pp 787

the drug In the midafternoon all greens and grain were removed from one half of the cages, and these animals, called unfed, received no food during the next twenty-four to thirty hours All animals had access to water On the morning of the injection, usually at about 10 a m, the “fed” group was given a copious amount of greens All animals received injections three or four hours later Autopsies have shown that the stomachs and small intestines of the “unfed” animals were

TABLE 2—Extent to Which Age of Guinea Pigs and Food Intake Were Related to the Development of Intestinal Lesions

| Age* of Guinea Pigs | Fed or Unfed† | Substance Injected                             | Dose, Cc per Kg | Animals        |              | Total Number of Injections | Deaths Due to |                 |          |                 |
|---------------------|---------------|--|-----------------|----------------|--------------|----------------------------|---------------|-----------------|----------|-----------------|
|                     |               |  |                 | 1st Injection# | 2d Injection |                            | Toxicity      | Intussusception | Volvulus | Fecal Impaction |
| Adult               | Fed           | Oily medium §<br>2% pilocarpine in oily medium | 15              | 27             | 21           | 48                         | 0             | 0               | 0        | 0               |
|                     |               |  | 15              | 27             | 21           | 48                         | 18            | 0               | 1        | 0               |
|                     | Unfed         | Oily medium<br>2% pilocarpine in oily medium   | 15              | 26             | 22           | 48                         | 0             | 0               | 0        | 0               |
|                     |               |  | 15              | 26             | 22           | 48                         | 7             | 2               | 0        | 0               |
| Young               | Fed           | Oily medium<br>2% pilocarpine in oily medium   | 15              | 50             | 46           | 96                         | 0             | 0               | 0        | 0               |
|                     |               |  | 15              | 50             | 27           | 77                         | 35            | 3               | 0        | 1               |
|                     | Unfed         | Oily medium<br>2% pilocarpine in oily medium   | 15              | 49             | 39           | 88                         | 0             | 0               | 0        | 0               |
|                     |               |  | 15              | 49             | 28           | 77                         | 14            | 12              | 0        | 1               |

\* The adult guinea pigs were over 7 months of age, the young ones ranged in weight from 200 to 450 Gm and were still growing rapidly  
† The “unfed” guinea pigs had only water for twenty four hours preceding the injection of the drug The “fed” animals were given mixed grains and lettuce greens as usual, being fed the greens at about 10 a m and given the injection in the midafternoon  
§ This was 5 per cent beeswax in liquid petrolatum  
# One week elapsed between injections

TABLE 3—Analysis of the Data on Intussusception Given in Table 2

| Groups of Animals       | Substance Injected*                      | Number of Injections Received by Animals Surviving Toxicity | Intussusceptions Observed |    | Chi (Deviation Divided by Standard Deviation) |
|-------------------------|--|---|---------------------------|----|---|
|                         |  |   | No                        | %  |   |
| Fed                     | Pilocarpine hydrochloride in oily medium | 72  | 3                         | 4  | } 2.1   |
| Unfed                   | Same                                     | 104   | 14                        | 13 |   |
| Adult                   | Pilocarpine hydrochloride in oily medium | 71  | 2                         | 3  | } 2.5   |
| Young                   | Same                                     | 105   | 15                        | 14 |   |
| All controls            | Oily medium                              | 280   | 0                         | 0  | } 5.7   |
| All pilocarpine treated | Pilocarpine hydrochloride in oily medium | 176   | 17                        | 10 |   |

\* The dose for all animals was 15 cc per kilogram injected subcutaneously, 2 per cent pilocarpine hydrochloride was used

empty at this time, whereas the stomachs of the “fed” animals were filled with food and the small intestines contained partly digested food In the colons of the “unfed” and the “fed” animals were feces, usually more abundant in the latter After the injection, the animals were given food at about 7 p m, but, because of the toxic effects of the drug, were never observed to eat until the next day  
The animals were given injections of the oily medium only on the first and fourteenth days of the experiment, during these mock injections the groups were treated as during the pilocarpine injections of the seventh and twenty-first days The details and results of the experiments are shown in tables 2 and 3

It was evident that young animals were somewhat more susceptible to intussusception than adult animals, since in these experiments intussusception developed in about 14 per cent of the young ones and only 3 per cent of the adults

Lack of feeding also increased the tendency toward development of this lesion, and 13 per cent of the "unfed" showed intussusception, compared with 4 per cent of the animals adequately fed the morning of the injection. The highest incidence of intussusception, 21 per cent, was found in the young, "unfed," pilocarpine-stimulated animals

A rather surprising increase in toxicity was noted in the animals fed before the injection of the pilocarpine salt, and it was evident that starving the animals for twenty-four hours before the injection of the drug reduced the mortality from toxicity

A summation of the data of tables 1, 2 and 3 is shown in table 4. In these experiments 22 intussusceptions, 3 volvuli and 6 fecal impactions occurred. All were considered to be the cause of death. The incidence of each of these lesions among the pilocarpine-treated guinea pigs is greater than might be expected by chance alone. It seems that the same stimulus may, under different conditions in the same

TABLE 4—Summary of Data of Tables 1, 2 and 3

| Groups of Animals   | Substance Injected                       | Number of Injections Received by Animals Surviving Toxicity | Number of Lesions Observed |           |                  | Chi (Deviation Divided by Standard Deviation) |
|---------------------|--|---|----------------------------|-----------|------------------|---|
|                     |  |   | Intus suscep tion          | Vol vulus | Fecal Impac tion |   |
| Controls            | Oily medium or saline solution           | 483   | 0                          | 0         | 0                |   |
| Pilocarpine treated | Pilocarpine hydrochloride in oily medium | 344   | 21                         | 3         |                  | 5.5   |
|                     | or in saline solution                    |   |                            |           | 6                | 2.3   |
|                     |  |   |                            |           |                  | 2.9   |

guinea pigs, or in different guinea pigs, because of anatomic or other conditions not understood, produce one of the three lesions

Spontaneous occurrence of intussusception, volvulus or fecal impaction has not been observed in these animals in this laboratory

*Present Technic of Production of Intussusception*—At the present time the following method is used to produce these lesions in guinea pigs

*Materials* Young guinea pigs weighing under 400 Gm are used. A 2 per cent suspension of pilocarpine hydrochloride in an oily medium (5 per cent beeswax in liquid petrolatum) is injected

*Method* 1 Weigh animals after feeding. 2 Deprive of food for twenty-four hours. 3 Inject subcutaneously 15 cc per kilogram of the suspension of pilocarpine hydrochloride. 4 Observe animals for more than one week. If desired, the survivors can be given further injections after one or two weeks

Probable results (based on 92 injections given to 64 animals): deaths due to toxicity, 15 per cent, to intussusception, 17 per cent, to volvulus, 2 per cent, to fecal impaction, 3 per cent

#### DESCRIPTION OF LESIONS

*Intussusception*—In the experiments listed in tables 1, 2 and 3, and in experiments of a preliminary nature not listed in these tables, a total of 32 intussusceptions were observed in guinea pigs. Of these, 10 were in the jejunum, 14 in the ileum, 2 of the ileum into the cecum (ileocecal) and 6 in the colon

The 24 intussusceptions of the small intestine varied in length from 2 to 12 cm, and the longer lesions were convoluted, often looking like a thick helical spring. In every instance the upper portion of the bowel had invaginated into the lower, dragging in the mesenteric vessels. The intussusceptum was always swollen, edematous and hemorrhagic, and its distal two thirds usually necrotic. The intussusciptens was dilated, edematous and often hyperemic as well. The portion of the intestine above the lesion was dilated with gas or fluid, and that below contracted and empty of food, though frequently containing bloody material. In 6 instances the colon contained from 5 to 15 Gm of bloody, tarry stool, obviously indicating a rather severe hemorrhage from the intussusception.

The two ileocecal lesions were about 6 cm in length, and the intussusceptum was hemorrhagic, edematous and necrotic. In 1 instance there was much bloody material in the cecum.

In the 6 instances of colic intussusception the invagination occurred in the descending colon, and in 2 instances the descending colon invaginated into the rectum, and the necrotic, hemorrhagic intussusceptum protruded for about a centimeter beyond the anal orifice. Three of the 6 animals had a large fecal impaction above the point of obstruction, and 2 of these died of generalized peritonitis following perforation of the colon.

It was frequently possible to make an antemortem diagnosis of intestinal obstruction in animals with intussusception, volvulus or fecal impaction, since the animals refused all food, sat dejectedly with backs humped and hair erect, lost weight rapidly, became weak, and died. Some passed into a condition resembling shock with surprising rapidity.

The duration of life of animals with intussusception varied considerably. When the lesion was in the colon, the period of survival after the injection of the drug varied from one and a half to seven days, with an average survival of four days. Of the 26 animals which had lesions in the jejunum, the ileum or the ileocecal region, 3 died during the first twelve hours, probably from a combination of the toxicity of the drug and the effects of the intussusception, in these the intussusceptum was already hemorrhagic and edematous, and the portion of the bowel above distended. The remaining 23 survived the period of obvious toxicity, 15 dying in the twelve to thirty-six hour period, and 8 thereafter, some living as long as four days.

*Volvulus*—Three examples of volvulus were observed. Two were located in the small intestine, and one in the hepatic flexure of the colon. In one of the lesions in the small intestine the total rotation was 1080 degrees, and the bowel was hemorrhagic and edematous. In the other lesion of the small intestine the rotation was almost as great, and involved 70 cm of intestine, which contained 40 cc of fluid, a not inconsiderable fluid loss for so small an animal. The rotation of the volvulus in the colon was only 180 degrees, yet it produced obstruction. These animals survived from one to three days, the guinea pig having the obstruction in the colon living the longest.

*Fecal Impaction*—In 6 animals death was caused by fecal impaction of the colon. In these the terminal portion of the descending colon was empty and contracted, and the transverse and ascending colon were solidly packed with hard fecal material, often forming a mass measuring about 1 cm in diameter and completely obstructing the bowel. There was usually ulceration of the mucosa overlying the fecal mass, and in 5 instances perforation of the colon occurred, resulting in peritonitis. Two animals with this lesion lived only one day, dying of perforation of the colon and peritonitis. The other 4 lived from three to six days.

## COMMENT

The mechanism of the production of intussusception in these guinea pigs is not fully understood. Following the administration of pilocarpine hydrochloride, there is marked hyperperistalsis of the intestine and hypersecretion of the salivary glands, the pancreas and perhaps the intestinal glands as well. With large doses, such as were used in these experiments, severe dehydration and prostration are also observed. Of all these factors hyperperistalsis seems the most likely cause of intussusception.

The neural and muscular mechanisms involved in intussusception have been studied,<sup>5</sup> and the process probably develops as follows:

The peristaltic rush consists of an advancing wave of contraction preceded by a wave of relaxation. As long as inhibition precedes contraction, no harm can result, but if, when the wave of contraction reaches the lower end of the ileum, the inhibition phase is not transmitted, due to failure of the inhibitory mechanisms, the strong contraction of the peristaltic rush may carry a portion of the gut into the distal segment as an invagination, thus initiating the intussusception.<sup>12</sup>

Many theories have been advanced to explain the high incidence of intussusception in children under 2 years of age, and the fact that the vast majority of cases are of the ileocecal or ileocolic varieties. In an extensive monograph Perrin and Lindsay<sup>13</sup> analyzed 400 cases of intussusception. Seventy-eight per cent occurred under the age of 2 years, 70 per cent under 1 year, and 50 per cent between the fifth and the ninth month of life. The authors observed that the disease was slightly more common in the spring but had no relation to diarrhea. Of the types observed, 46.5 per cent were ileocecal, 37.6 per cent ileocolic, 10.1 per cent enteric and 5.6 per cent colic. In their discussion of the causation of the lesion they stated that perverted peristalsis alone could not account for the majority of the intussusceptions but that they believed that "in the case of ileocecal, ileocolic, and enteric types the majority of intussusceptions are caused by inflammatory swelling of lymphoid tissue." They suggested that many gastrointestinal disturbances cause swelling of lymphoid tissue, and so predispose to intussusception.

The great prominence of the valve, and the narrow lumen of both ileum and—especially—colon during the first year of life is probably an accessory factor in the production of these two forms (ileocecal and ileocolic), for any swelling of either the valve or the lymphoid tissue would readily come into contact with the segment of gut immediately below the swelling, and be treated as a foreign body.

They expressed the belief that the age incidence could be explained by the presence of a maximal amount of lymphoid tissue in early life, and explained the common anatomic site of the lesion by the quantity of

<sup>12</sup> Kuntz, A. *The Autonomic Nervous System*, ed. 3, Philadelphia, Lea & Febiger, 1945, pp. 504-505.

<sup>13</sup> Perrin, W. S., and Lindsay, E. C. *Brit. J. Surg.* 9: 46, 1921.

lymphoid tissue about the ileocecal valve. They suggested that fat babies have most lymphoid tissue, thus explaining the high incidence of the disease in these healthy, well nourished children. The views of Perrin and Lindsay have found support,<sup>14</sup> and Wakeley and Atkinson<sup>15</sup> have gone so far as to state "There can be no doubt that this increase of lymphoid tissue in the lowest part of the ileum acts as a foreign body and causes increased and irregular peristalsis of the bowel which, at times, culminates in an intussusception."

It seems likely that in infants and young children there is some anatomic peculiarity of the ileocecal valve which explains the great frequency of intussusception at this site. The theory advanced by Perrin and Lindsay<sup>13</sup> may be quite valid, but objections may be raised to this theory that "hyperplastic lymphoid tissue in the terminal ileum and ileocecal valve" produces intussusception. Harris and his associates<sup>16</sup> stated that in man lymphoid tissue reaches its maximum development at about the twelfth year, certainly long after the greatest incidence of intussusception. Wangenstein<sup>14</sup> has pointed out that if Peyer's patches of lymphoid tissue followed the same growth as other lymphoid tissue, there would be a large number of cases of intussusception occurring in the early teens. Other factors, such as the relation of the diameter of the ileum to the mass of lymphoid tissue in its wall, may operate, however, and help explain why intussusception is so common in the very young. It is also possible that the projection of the ileocecal valve into the cecum<sup>14</sup> and the failure of proper passage of peristaltic waves at this site<sup>12</sup> are of importance.

In our experiments, at least three factors were of importance in the production of intussusception. The first of these was the effect of a large dose of pilocarpine hydrochloride on the intestine, which presumably caused severe hyperperistalsis and spasm, the second, the age of the animal, the lesion occurring more frequently in young animals than in old, and finally, the presence or the absence of food in the gastrointestinal tract, the latter increasing the incidence of the lesion.

That hyperperistalsis may precede the development of intussusception has been shown in animals by Propping,<sup>7</sup> and by Watts and Fulton.<sup>5a</sup> Many pathologists and others have observed the formation of small intussusceptions during the hyperperistalsis which occurs in the intestines immediately after death. Our observation that excessive pilocarpine stimulation of the intestine may at times result in intussusception is, then, in accord with that of other workers.

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14 Montgomery, A. H., in Brennemann, J. *Practice of Pediatrics*, Hagerstown, Md., W. F. Prior Company, Inc., 1945, vol. 3, chap. 6, pp. 14-21.

15 Wakeley, C. P. G., and Atkinson, F. R. B. *Brit. J. Child Dis.* **35**: 241, 1938.

16 Harris, J. A., Jackson, C. M., Patterson, D. G., and Scammon, R. E. *The Measurement of Man*, Minneapolis, University of Minnesota Press, 1930.

Veterinarians<sup>2</sup> have suggested that hyperperistalsis precedes the development of intussusception in animals, but clinicians are not in agreement concerning the relation of hypermotility of the intestine to the onset of this lesion in man. Perrin and Lindsay<sup>13</sup> mentioned only "perverted peristalsis" as a possible cause. Others<sup>17</sup> also have felt that hyperperistalsis is not an important factor. Nevertheless, in a large proportion of the cases the intussusception occurs at the time of weaning from the breast, and this change in dietary regimen may occasion spasm of the bowel.<sup>12</sup> Also Perrin and Lindsay<sup>13</sup> and others<sup>15</sup> have observed that teething, inadequacy of mothers' milk and change in diet may be factors in the production of intussusception between the fifth and the ninth month, when it occurs in half of the cases. Although these authors attributed this incidence to swelling of lymphoid tissues occasioned by these disturbances, it is as reasonable that hunger and subsequent hyperperistalsis may play a role in the genesis of the disease at this age.

The fact that intussusception is more common in young animals has been observed by veterinarians,<sup>2</sup> and over 70 per cent of human patients have been children under 2 years, and over half of those were under 1 year of age.<sup>18</sup> Our experiments have shown that young guinea pigs are more susceptible to this lesion than older animals, a fact which is in accord with clinical observations.

The cause of the increased susceptibility of young animals to intussusception is still poorly understood. In the very young, the reflexes of the gastrointestinal tract are poorly coordinated,<sup>19</sup> and hyperperistalsis may be more common than in the old. In infants and young children there is excessive lymphoid tissue in the terminal part of the ileum and the ileocecal valve,<sup>20</sup> with protrusion of the ileocecal valve into the cecum.<sup>14</sup> It is evident that although the site in young children and infants may be explained on an anatomic basis, the increased susceptibility may be dependent in part on physiologic factors, such as hyperperistalsis and poor coordination of intestinal peristalsis, or on anatomic defects common to young animals, such as softness of tissues and weakness of musculature.

Rather unexpected findings were the increased incidence of intussusception and decreased incidence of toxicity in animals deprived of food for twenty-four hours before the injection of pilocarpine hydrochloride. The cause of these phenomena is not clear, but they do not seem to be directly related to each other, since in the experiments of table 1 the number of deaths from toxicity of the "fed" animals stimulated with pilocarpine was about the same (20 per cent) as that of the "unfed"

17 Tumen<sup>1b</sup> Montgomery<sup>14</sup> Wakeley and Atkinson<sup>15</sup>

17 Tumen<sup>1b</sup> Montgomery<sup>14</sup> Wakeley and Atkinson<sup>15</sup>

18 Wangenstein<sup>12</sup> Perrin and Lindsay<sup>13</sup>

19 Fraser, J. Brit. M. J. 1 359, 1926 Kuntz<sup>12</sup>

20 Perrin and Lindsay<sup>13</sup> Montgomery<sup>14</sup> Wakely and Atkinson<sup>15</sup>

animals of table 2 and 3 (18 per cent), yet the incidence of intussusception in the latter groups is considerably higher. It is possible that food acts as a bolus and prevents intussusception by interfering with maximal contraction or by making the reflex pattern of peristalsis more uniform.

It is difficult to obtain sufficient data from clinical investigations of intussusception to be certain that an empty gut is more apt to undergo intussusception than one that is full. The observation that the disease is most common at the time of inadequacy of mother's milk, weaning and teething<sup>21</sup> suggests that the empty state of the intestine may be of importance.

That fecal impaction and volvulus occur in addition to the more frequent intussusception does not seem accidental (see table 4). Volvulus can probably be explained, like intussusception, on the basis of hyperperistalsis, and, indeed, both lesions may be caused by errors of motility of the intestine. It is possible that the fecal impaction is similar in its origin, perhaps under the influence of pilocarpine one portion of the colon becomes spastic passing no feces, while the hyperperistaltic portion packs feces in above the site of spasm so firmly that, when the effects of the drug have disappeared, the intestine may be unable to move the fecal mass. Other factors, however, such as dehydration, may play a role in the causation of fecal impaction.

#### SUMMARY AND CONCLUSIONS

Thirty-two intussusceptions have been observed in guinea pigs following the subcutaneous injection of pilocarpine hydrochloride. The disease is more easily induced in young than in old animals, and the incidence of the lesion is increased by withholding food for the twenty-four hours preceding the injection. It is concluded that immaturity and an empty intestinal tract predispose these animals to intussusception. The relationship of these findings to clinical observations of this disease is discussed.

The occasional occurrence of volvulus and fecal impaction in animals stimulated with pilocarpine has been observed, and the action of pilocarpine, presumably by increasing the peristaltic and other movements of the intestines, is probably a factor in the production of these lesions, as well as of intussusception.

An effective means of producing such lesions is to select and weigh young guinea pigs, hold them without food for twenty-four hours, then inject subcutaneously 1.5 cc per kilogram of 2 per cent suspension of pilocarpine hydrochloride in a 5 per cent beeswax-liquid petrolatum mixture. About one sixth of the animals will die of toxicity and a similar number of intussusception, about 3 per cent will die of fecal impaction and about 2 per cent of volvulus.

21 Wangenstein<sup>1a</sup> Perrin and Lindsay<sup>13</sup> Wakely and Atkinson<sup>15</sup>



# DIABETES AND UREMIA DIAGNOSED AT AUTOPSY BY TESTING CEREBROSPINAL FLUID AND URINE

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LESIONS such as degeneration of islet cells in the pancreas, deposition of glycogen in the renal tubules and intercapillary glomerulosclerosis will frequently confirm the clinical diagnosis of diabetes mellitus. However, whether or not a person died from diabetic acidosis is a question which cannot be answered from anatomic evidence. It is this problem which confronts the pathologist in cases of sudden death when clinical data are lacking and necropsy fails to reveal significant changes. In such cases a pathologic diagnosis of diabetic coma can be established by testing suitable postmortem material for sugar and acetone.

Not quite analogous is the situation in cases of uremic coma, as gross renal changes are generally obvious. However, again it is difficult to draw conclusions from anatomic changes as to the functional damage and to determine their role in the causation of death, especially in the presence of multiple lesions of other organs. Here, the degree of nitrogen retention in postmortem material will elucidate the case and permit a logical interpretation of the relative importance of anatomic findings.

It is the purpose of this paper to describe rapid tests for sugar, acetone and urea to be performed on cerebrospinal fluid at the autopsy table, to show the importance of postmortem analysis of the urine and to point out the value of these procedures as aids in the postmortem diagnosis of diabetes and uremia.

## TECHNIC OF OBTAINING CEREBROSPINAL FLUID

The cerebrospinal fluid used for testing is obtained by cisternal puncture and may be used directly if clear. Maximal bending of the head, careful direction of the needle (preferably an 18 gage Barker needle) and gentle suction will generally avoid blood contamination. The latter, as well as an increase of cerebrospinal protein, necessitates protein precipitation.

## DESCRIPTION OF TESTS MADE WITH CLEAR CEREBROSPINAL FLUID OF NORMAL PROTEIN CONTENT

*Technic of Sugar Test*—This technic is an adaptation of the "clinitest"<sup>1</sup> method for urinary sugar. Fifteen drops of cerebrospinal fluid are delivered from an eye

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<sup>1</sup> This commercial preparation is recommended only because of its availability and the practicability of its use.

dropper into a tube of  $\frac{5}{8}$  inch (1.6 cm) diameter, such as a Wassermann tube, and a "clinitest" tablet is added. If glucose is not increased, the reaction mixture remains blue after effervescence ceases. A green or yellow color indicates an increase of glucose which may be roughly estimated in percentage by comparing the color with the "clinitest" color chart. The percentage thus estimated and expressed in milligrams per hundred cubic centimeters should be divided by 3, as the original method for urine is based on a 1:3 dilution.

**Comment.** Some simple precautions should be observed in performing the test. In dispensing the drops the eye dropper must be held vertical to insure a standard size of drops. During effervescence vigorous agitation is necessary in order to break up the froth due to protein and to obtain complete solution of the tablet. A mauve shade caused by the biuret reaction indicates excessive protein content and may require repetition of the test after the protein has been precipitated as described in a later section. If the cerebrospinal fluid removed from a refrigerated body is very cold, the heat generated by the dissolution of the tablet is insufficient to insure complete reduction of the copper hydroxide and slight warming is necessary. This is done by holding the tube containing the 15 drops of cerebrospinal fluid under the warm water tap. As tablets corrode easily under the moisture of the air (evidenced by a partially blue instead of a uniform gray appearance), it is expedient to keep them mixed with granulated calcium chloride in a wide mouth, screw-capped bottle.

**Technic of Acetone Test.**—This technic is an application of the dry powder method for urinary acetone devised by Laughlen<sup>2</sup> and is performed by placing a drop of cerebrospinal fluid on a small amount of powder<sup>3</sup> heaped on paper. In the absence of acetone the color of the moistened powder will be a gray-yellowish or lavender which fades quickly. A pink color stable for at least three minutes indicates presence of acetone.

**Comment.** As demonstrated by the Dumm-Shipley<sup>4</sup> acetone test in serum, protein does not interfere with the reaction, nor does a slight amount of blood. In the presence of heavy blood contamination, the cerebrospinal fluid may be centrifuged or precipitated.

**Technic of Urea Test.**—The test is based on the yellow color reaction of urea with Ehrlich's aldehyde reagent, first described by Weltmann and Barrensen<sup>5</sup> and modified as a spot test in blood by Naumann, Plotz and Reich<sup>6</sup>. The performance of the test is identical with that described by me<sup>7</sup> for detecting urobilinogen in cerebrospinal fluid, viz., adding of 2 drops of aldehyde reagent<sup>8</sup> to 2.5 cc of cerebrospinal fluid in a 10 by 75 mm tube. The resulting yellow color is viewed through the height of the fluid column against a white background and compared

2 Laughlen, G. F. *Canad. J. Med. Technol.* 5:3, 1943.

3 "Acetone test Denco" was used. Dumm and Shipley's formula is equally satisfactory.

4 Dumm, R. M., and Shipley, R. A. *J. Lab. & Clin. Med.* 31:1162, 1946.

5 Weltmann, O., and Barrensen, H. K. *Klin. Wochenschr.* 1:1100, 1922.

6 Naumann, H. N., Plotz, M., and Reich, N. E. *J. Lab. & Clin. Med.* 28:335, 1942.

7 Naumann, H. N. *Proc. Soc. Exptl. Biol. & Med.* 65:72, 1947.

8 1% p-dimethylaminobenzaldehyde "Kodak" dissolved in ice cold, concentrated hydrochloric acid and kept in a dropping bottle.

with a standard solution<sup>9</sup> equivalent to 200 mg of urea per hundred cubic centimeters in an identical tube. Normally or in the presence of moderate nitrogen retention the yellow reaction is less than that of the standard, while a color intensity greater than that of the standard is found in cases of severe renal damage.

**Comment** If an orange or pink color develops, due to cerebrospinal urobilinogen, the test is repeated after first adding a drop of formaldehyde solution U S P which inhibits the urobilinogen but not the urea reaction. The fluid should then be examined without delay as the formaldehyde solution causes fading of the yellow color. If slightly contaminated with blood, the cerebrospinal fluid should be centrifuged, but protein precipitation is necessary if a larger amount of blood or of protein is present.

#### TECHNIC OF TESTS IN THE PRESENCE OF EXCESSIVE CEREBROSPINAL BLOOD OR PROTEIN

Slight blood contamination interferes with the urea test but not with the sugar and acetone tests. Heavy blood contamination or large amounts of cerebrospinal protein require protein precipitation.

For the precipitation of protein, an adaptation of the colloidal iron method of Rona and Michaelis<sup>10</sup> has been found most suitable and is performed by mixing 10 drops of 5 per cent dialyzed iron "Merck" with 5 cc of cerebrospinal fluid, adding a pinch of sodium chloride and filtering through a small filter. The water-clear filtrate is then subjected to the tests described in the foregoing section.

**Comment** In the presence of large amounts of blood and protein the number of drops of iron solution may have to be increased to 15 or 20. The dilution of the cerebrospinal fluid resulting from the addition of the iron solution need not be considered in the rough estimates described.

#### POSTMORTEM ANALYSIS OF URINE

Urine was obtained by catheterization or by puncturing the exposed bladder. Included in the routine analysis were determinations of the specific gravity and the reaction, tests for albumin (sulfosalicylic acid method), sugar (the "clintest" method or Benedict's), acetone (Legal's or the dry powder method), urobilinogen (Ehrlich's test) and microscopic examination.

#### RESULTS

Tables 1 and 2 give a survey of representative cases selected from a larger material<sup>11</sup>. In evaluating these results it should be borne in mind that the tests described are approximations based on qualitative tests and not analytic procedures. The figures given are intended to indicate ranges of increase sufficient to establish a diagnosis of diabetes or uremia.

<sup>9</sup> The usual icterus index standard containing 0.01 per cent potassium bichromate and 0.1 per cent concentrated sulfuric acid corresponds in color to that produced in a solution of 200 mg urea per hundred cubic centimeters under the conditions of the test.

<sup>10</sup> Rona, P., and Michaelis, L. *Biochem Ztschr* 7:332, 1908, 14:479, 1908.

<sup>11</sup> Naumann, H. N. To be published.

Of the first 2 subjects with high cerebrospinal sugar and acetone (table 1), the second died in coma Shortly before death this patient

TABLE 1—*Postmortem Tests for the Sugar and Acetone Contents of Cerebrospinal Fluid and Urine*

| Sub<br>ject | Hours<br>Post<br>Mortem | Age,<br>Sex | Cerebrospinal Fluid |              |   | Urine |              | Chief Clinical and Anatomic<br>Diagnosis  |
|-------------|-------------------------|-------------|---------------------|--------------|---|-------|--------------|---|
|             |                         |             | Rapid Tests         |              | Sugar,<br>Quantita-<br>tive<br>Determination† | Sugar | Ace-<br>tone |   |
|             |                         |             | Sugar*              | Ace-<br>tone |   |       |              |   |
| 1           | 13½                     | 72<br>♂     | 333+                | +            | 402   | 0.25% | +            | Arteriosclerotic heart disease<br>diabetes (mild)                                 |
| 2           | 7                       | 28<br>♂     | 167—<br>333         | ++           | 222   | 4+    | 4+           | Diabetic coma, patient re-<br>ceived 100 units of insulin<br>shortly before death |
| 3           | 3                       | 80<br>♀     | 167                 | ±            | 146   | 0     | 0            | Pneumonia, adrenal hemor-<br>rhage, thrombophlebitis                              |
| 4           | 1                       | 65<br>♀     | 83—<br>167          | 0            | 131   | 0     | 0            | Pancreatitis, pneumonia,<br>arteriosclerosis                                      |
| 5           | 22                      | 75<br>♀     | 0                   | 0            | 47  | —     | —            | Cirrhosis of the liver, portal<br>thrombosis, intestinal hem-<br>orrhage          |
| 6           | 5                       | 71<br>♂     | 0                   | 0            | 19  | 0     | 0            | Diabetes, hypertension, pan-<br>creatic fibrosis                                  |

\* The figures indicate milligrams per hundred cubic centimeters, a plus sign following a number denotes "more than" and a minus sign "less than"

† The Folin Wu blood sugar method was used in duplicate determinations

TABLE 2—*Postmortem Tests for the Urea Content of Cerebrospinal Fluid and Urinalysis*

| Sub<br>ject | Hours<br>Post<br>Mortem | Age,<br>Sex | Cerebrospinal<br>Fluid |   | Urine               |             |                       | Chief Clinical and Anatomic<br>Diagnosis                           |
|-------------|-------------------------|-------------|------------------------|---|---------------------|-------------|-----------------------|--|
|             |                         |             | Rapid<br>Urea<br>Test* | Urea,<br>Quantitative<br>Determination† | Specific<br>Gravity | Albu<br>min | Sedi<br>ment          |  |
|             |                         |             |                        |   |                     |             |                       |  |
| 7           | 1½                      | 73<br>♂     | 200+                   | 428                                     | —                   | —           | —                     | Nephrosclerosis, severe, arteriosclerosis, pneumonia               |
| 8           | 2½                      | 37<br>♂     | 200+                   | 287                                     | 1.017               | +           | Many casts            | Nephrosis, dementia paralytica, malaria treatment                  |
| 9           | 14                      | 75<br>♂     | 200                    | 268                                     | 1.016               | ++          | Many casts, red cells | Hypernephroma, obstructive jaundice (stone), myocardial fibrosis   |
| 10          | 8                       | 40<br>♂     | 150—<br>200            | 377                                     | 1.021               | +           | —                     | Pulmonary tuberculosis fatty degeneration of heart, liver, kidneys |
| 11          | 5                       | 52<br>♀     | 50—<br>100             | 48                                      | 1.025               | ±           | Many pus cells        | Glioblastoma multiforme, pneumonia nephrosclerosis                 |
| 12          | 13                      | 76<br>♀     | 0                      | 24                                      | 1.021               | 0           | Some pus cells        | Hypertension, nephrosclerosis, fracture of femur                   |

\* The estimates were obtained by comparing the colors with standards containing 50, 100, 150 and 200 mg per hundred cubic centimeters

† My direct nesslerization method (Naumann, H N J Lab Clin Med 26 405, 1940) was used in duplicate determinations

had received 100 units of insulin, which apparently lowered the sugar from the expected extreme level The first patient's condition was clinically diagnosed as arteriosclerotic heart disease, but the true cause

of death was diabetic coma as revealed by the chemical examination. On the other hand, the low sugar in the case of subject 6, known to be diabetic indicates that the diabetes was well controlled and could not have played a role in the causation of death. Subjects 3 and 4 illustrate secondary effects on the carbohydrate metabolism due to adrenal hemorrhage and acute hemorrhagic pancreatitis, respectively. The urinary findings corroborated those in the cerebrospinal fluid with the exception that in the case of subject 1 urinary sugar and acetone reactions were of a lesser degree than expected.

The high urea levels of the first 3 subjects in table 2 point to severe renal damage and to the renal changes as the primary factor or at least a main contributory factor in the causation of death. The low urea values of the last 2 subjects, probably also those in case 10, again preclude the possibility that the renal changes found at autopsy were of pathognomonic significance. The urinary findings agreed well with these results.

#### COMMENT

There are relatively few reports of postmortem chemistry in the literature, and those concern mainly the blood, with confusing results. While Bigwood<sup>12</sup> denied any value of postmortem chemical analysis, it was found by Polayes, Hershey and Lederer<sup>13</sup> that, of all routine chemical procedures, the determination of creatinine alone gives values comparable with those obtained during life. Similar but more limited conclusions were reached by Hamilton<sup>14</sup>. Wuchermann<sup>15</sup> found an agonal rise of the nonprotein nitrogen amounting to about 35 mg per hundred cubic centimeters with further postmortem increases and warns against any attempt to diagnose clinical uremia from high postmortem nitrogen values. Pozzan and Lenaz,<sup>16</sup> on the other hand, stated that postmortem urea is generally elevated but that values in excess of 300 mg per hundred cubic centimeters of the blood and 200 mg per hundred cubic centimeters of the cerebrospinal fluid are obtained only for persons who died from renal diseases. Riva<sup>17</sup> likewise concluded that a postmortem blood urea level of 200 mg or more per hundred cubic centimeters in slow death or of 100 mg or more in sudden death indicated uremia during life, while a postmortem urea concentration of 100 mg or less per hundred cubic centimeters precluded uremic death.

12 Bigwood, E. J. *Ann de méd lég* **10** 284, 1930

13 Polayes, S. H., Hershey, E., and Lederer, M. *Arch Int Med* **46** 283, 1940

14 Hamilton, R. C. *Arch Path* **26** 1135, 1938

15 Wuchermann, F. *Ztschr f klin Med* **127** 491, 1935

16 Pozzan, A., and Lenaz, A. *Gior veneto sc med* **9** 848, 1935

17 Riva, G. *Helvet med acta (supp 12)* **1** 62, 1943

From studies on postmortem glycolysis Hamilton-Paterson and Johnson<sup>18</sup> concluded that postmortem sugar concentrations are low except in the blood of the vena cava and the right ventricle, owing to postmortem glycogenolysis in the liver and diffusion of sugar accumulated in the hepatic vein. By using blood of the left ventricle they obtained reliable results, and 200 mg of sugar or more per hundred cubic centimeters indicated antemortem hyperglycemia. These authors also found cerebrospinal fluid suitable for postmortem sugar determinations. Hill,<sup>19</sup> on the basis of carefully devised experiments, substantiated these findings and studied a large material of forensic cases with the result that conditions other than diabetes, such as asphyxia, intracranial hemorrhage and shock, were shown to cause marked hyperglycemia occasionally. Jetter and McLean<sup>20</sup> also recommended the use of left ventricular blood for postmortem sugar determinations. As far as post-mortem analysis of urine is concerned, no references could be found in the literature from 1927 to the present.

Chemical changes encountered from one to twenty-four hours after death may be the result of agonal fluctuations or may be due to post-mortem action of enzymes, such as glycolysis, alteration of cell permeability or persistent function of organs as shown by the postmortem glycogenolysis of the liver and by revival of postmortem kidneys and hearts performed by Wilbur<sup>21</sup> and by Kountz.<sup>22</sup> These processes are taking place under favorable conditions of temperature, which, according to Hamilton-Paterson and Johnson,<sup>18</sup> decreases slowly inside the body over many hours even in the refrigerator. Owing to the action of these various factors, the concentrations of sugar found in blood and tissues post mortem are lower, and those in urea higher, than during life. However, after allowing for such postmortem "normals," diagnostic conclusions can be drawn with certain reservations.

Interference from postmortem changes is less disturbing in chemical examinations of cerebrospinal fluid and of urine. While cerebrospinal fluid shows glycolysis, it is, by virtue of the blood-cerebrospinal fluid barrier, less subject to agonal and postmortem fluctuations of chemical constituents than the general circulation. Other advantages are the absence of clotting and the comparative freedom from cells and protein, permitting usually testing without protein precipitation, which, if necessary, can be performed easily and without undue dilution. Even less marked are the changes in urine within twenty-four hours after death.

18 Hamilton-Paterson, J. L., and Johnson, E. M. W. *J. Path. & Bact.* **50**: 473, 1940.

19 Hill, E. V. *Arch. Path.* **32**: 452, 1941.

20 Jetter, W. W., and McLean, R. *Am. J. Clin. Path.* **13**: 178, 1943.

21 Wilbur, D. L. *Proc. Staff Meet., Mayo Clin.* **4**: 335, 1929.

22 Kountz, W. B. *Ann. Int. Med.* **10**: 330, 1936.

There are generally desquamation of the bladder epithelium, some disintegration of cells and casts, bacterial growth, and presence of spermatozoa in male urine—changes which prevent the evaluation of slight albumin reactions and cause disappearance of some structures of the sediment. However, it is evident that positive findings in a postmortem analysis of urine will provide just as important information to the prosecutor as *intra vitam* examination does to the clinician. Glycosuria, acetonuria, albuminuria of marked degree and casts, red cells and pus cells in the sediment have the same significance after death as during life for the diagnosis of diabetes and renal damage with the same limitation that the degree of the urinary findings does not necessarily parallel the severity of the disease.

Numerous analyses of postmortem cerebrospinal fluid and blood have led me<sup>11</sup> to the conclusion that postmortem cerebrospinal glucose levels of 200 mg or more per hundred cubic centimeters, equivalent to 0.5 per cent of sugar or more, in the rapid test are evidence of severe diabetes mellitus provided intravenous dextrose treatment was not given shortly before death. Asphyxia, intracranial hemorrhage and shock, which, according to Hill,<sup>19</sup> may all be accompanied with marked hyperglycemia, are not likely to cause appreciable glycosuria if death occurred in less than half an hour, since a rise of cerebrospinal fluid sugar lags behind that of blood sugar in time and degree. The simultaneous presence of acetone and glucose levels of 200 mg or more per hundred cubic centimeters, furthermore, is evidence of diabetic coma which may be accepted as the primary cause of death. Cerebrospinal fluid acetone, as well as urinary acetone, without increased glucose may be found in conditions in which body fat is incompletely oxidized, as in starvation and vomiting. Postmortem cerebrospinal sugar levels of 80 to 170 mg per hundred cubic centimeters, corresponding to 0.25 to 0.5 per cent of sugar, in the rapid test will generally be due to diabetes as well, but may occasionally be caused by metabolic fluctuations during prolonged agony or by conditions influencing the endocrine balance, such as coronary thrombosis, the anoxia of extensive pneumonia or massive hemorrhage and diseases of the ductless glands other than diabetes. Postmortem cerebrospinal sugar levels of 50 mg or less per hundred cubic centimeters giving negative results with the rapid test indicate either absence of diabetes or good control of a known diabetes.

In contrast to the well defined significance of the postmortem glucose concentrations of cerebrospinal fluid, it is much more difficult to interpret urea values. A majority of postmortem cerebrospinal fluids show in the presence of minor renal lesions urea levels between 60 and 190 mg per hundred cubic centimeters, which probably are due to agonal changes. Low urea figures, less than 100 mg per hundred cubic centimeters, are, therefore, more significant, and the rapid test will, thus, be of special

value as an exclusion test for renal insufficiency in the presence of pathologic conditions of the kidney. Postmortem urea levels of more than 200 mg per hundred cubic centimeters are comparatively rare and were found in my series only in the presence of marked renal changes. Nevertheless, conclusions should be drawn with caution unless extrarenal azotemia can be excluded and confirmation can be obtained from postmortem urinary findings. Under these conditions postmortem cerebrospinal urea levels of more than 200 mg per hundred cubic centimeters in the presence of renal disease may be accepted as evidence of renal insufficiency severe enough to constitute a major factor in the causation of death.

#### SUMMARY

Rapid tests for the sugar, acetone and urea contents of cerebrospinal fluid which may be conveniently performed at the autopsy table have been described and their value illustrated in the establishing of a diagnosis of diabetes and uremia after death.

The importance of postmortem analysis of urine in cases of diabetes and renal damage has been pointed out.

Postmortem cerebrospinal glucose levels of 200 mg or more per hundred cubic centimeters generally indicate severe diabetes, and simultaneous presence of acetone is evidence of diabetic coma which may be accepted as the primary cause of death.

Postmortem cerebrospinal urea levels of less than 100 mg per hundred cubic centimeters will rule out renal insufficiency in the presence of renal lesions. Urea levels of more than 200 mg under certain conditions may be accepted as evidence of uremia sufficiently severe to represent a major factor in the causation of death.

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## THE MURAL CELLS OF CAPILLARY HEMANGIOMAS

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BOSTON

IT IS customary to define capillary hemangioma as a tumor composed of vascular spaces of capillary or near capillary caliber. This definition should be taken literally. It implies that the lumens of the vascular units are quite small, resembling those of capillaries, it does not imply, in general, that these units are capillaries or vessels of similar caliber, in particular, it does not imply that the walls of these units are composed of elements identical with those of the capillary wall. In the tumor the structure of the wall of the unit need not at all, and rarely does, resemble that of a capillary vessel. While the predominant element composing the wall of a capillary is the endothelial cell, it is obvious that a large number, and frequently a majority, of the cells composing capillary hemangioma are not littoral in character. They have been delegated to a mural position.

To be sure, vascular units with endothelial or predominantly endothelial walls are found in these tumors, but their lumens usually exceed capillary caliber. These units often compose lobules in the periphery of the tumor, and suggest the presence of a higher intravascular pressure, with stretching of the walls, utilization of all tumor cells available, breakdown of the walls and fusion of adjacent units with formation of small scale cavernous portions.

What is the probable origin, the appearance, the nature and the fate of the mural cells of the capillary hemangioma?

In its less differentiated portions the tumor is composed of compact masses of oblong, spindle-shaped cells, fairly uniform in size, shape and staining qualities, with poorly defined cell boundaries and rare mitotic figures. There are occasional vascular spaces which may contain a few blood cells, but the vascular nature of the tissue is not evident everywhere. Numerous reticulum fibrils are present in many areas, forming a fine mesh enveloping single or small clusters of cells. The identification of stromal as distinguished from tumor cells is often impossible, though occasionally fibroblasts are seen from which the fibrils appear to originate.

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Where capillary lumens are more prominent the tumor cells may form one, two or more layers of spindle-shaped, fibroblast-like cells, not very different from those described in the foregoing paragraphs, around a central lumen containing red blood cells. In other areas, in vascular units of similar caliber, a difference in the appearance of the various components of the wall can be seen. Here the inner layer is composed of considerably flattened cells, with dark oblong flat nuclei and scanty, deep purplish cytoplasm which lines the lumen as a narrow dark rim. The remaining mural cells vary in appearance. They may have preserved their fibroblast-like appearance. In other places, however, both cytoplasm and nucleus are rounded out or have assumed a polyhedral shape. The cell boundaries are quite distinct, and the cytoplasm is water clear, with a few slate gray granules present. These cells have a distinct epithelioid character (figs 1 and 2). Mitotic figures are frequent in both epithelioid and "fibroblastic" mural cells. The mitotic figures of these cells by far outweigh those found in the endothelial cells.

The network of reticulum fibrils has been coarsened and condensed to form basement membrane-like lamellas around the vascular unit. In this manner the unit pattern becomes quite conspicuous with silver stains. Very rarely one can see portions of a reticulum fibril in between the mural cells, and often these portions appear continuous with the circular adventitial fibrils.

Only part of the wall of a vascular unit may show this differentiation of endothelial and mural cells. Small groups or clusters of epithelioid cells, set off against the deeply staining winding endothelial cytoplasm may be located in a wall which otherwise consists of cells of the "fibroblastic" type. This irregularity is striking and will be discussed in later paragraphs.

One feature of interest in units showing a distinct and complete differentiation of endothelial and mural epithelioid cells is the occasionally crenated outline of the lumen in paraffin sections. The endothelium appears to dip into mural crevices which are located between clusters of epithelioid cells, or, viewed differently, groups of mural cells appear to swell up, and bulge into the lumen of the unit, leaving the endothelium in the old position in places where this swelling is less evident. The endothelial nuclei may be located on the summits of these cushions of mural cells, they also may be located in the crevice, wedged into its exit. If the crevice is narrow, its nature may not be evident, an endothelial nucleus located in such a narrow cleft, between adjoining clusters of swollen mural cells, may appear like a leukocyte, or a large mononuclear cell migrating through the wall of the unit.

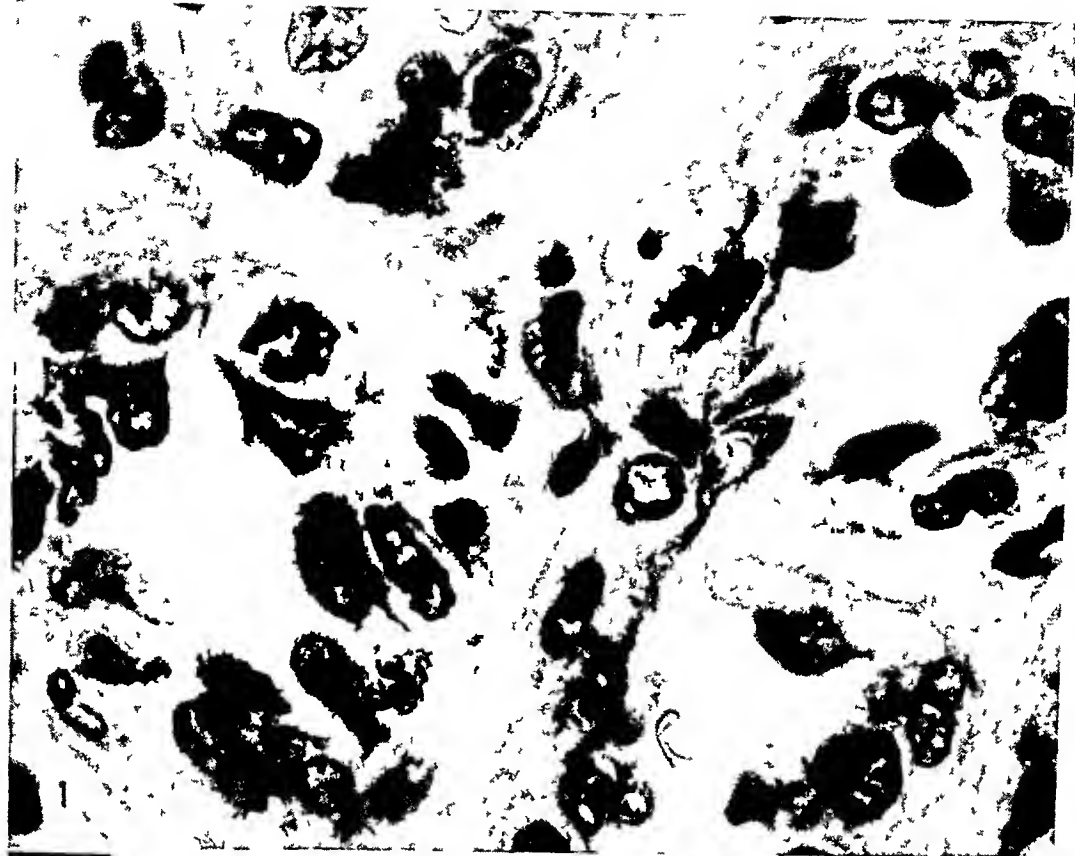


Fig 1—Several vascular units of a capillary hemangioma, showing the water-clear epithelioid mural cells differentiated from the endothelial cells with hyperchromatic cytoplasm. Hematoxylin-eosin,  $\times 1,060$

Fig 2—A portion of the wall of a vascular unit of a capillary hemangioma, showing clearly two endothelial cells each characterized by an elongated nucleus and a deeply staining cytoplasm. The endothelial cell in the center appears pushed into the lumen by a group of mural cells, while to the left an endothelial nucleus appears wedged into the exit of a crevice between two clusters of mural cells. Hematoxylin-eosin,  $\times 1,350$

One would expect the mural cells, with their numerous mitotic figures, to be the source of further vasoformative growth, and to be responsible for the deep infiltration and frequent recurrence seen in many tumors of this type. This expectation is not borne out by a study of the mural cells. The assumption of a mural position, it seems, is an irreversible change. Although further increase in thickness of the wall of the vascular unit occurs, this growth is out of proportion to the mitotic activity observed in the early stages.

With increase in thickness of the wall most vascular units display an increase of caliber and an increase of the local adventitial stroma. While mural cellular elements are abundant in the early stages, the further increase of thickness is largely due to formation of intercellular structures. The cells again are of fibroblastic appearance, and increasing numbers of collagen fibrils are laid down. In the later stages the units consist of a thin endothelium surrounded by a broad layer of collagenous fibrous tissue. On first glance, in sections stained with hematoxylin and eosin, these units may have the appearance of small to medium-sized arteries and veins. On close inspection, and in sections stained with phosphotungstic acid-hematoxylin and with aniline blue, the absence or deficiency of muscle cells and elastic fibers readily betrays their true nature.

#### HISTOLOGIC SEQUENCES IN THE FORMATION OR THE DEVELOPMENT OF MURAL NODULES

The walls of the larger vascular units frequently display areas of bulbous thickening. These mural nodules may reach considerable size, and their diameter may equal that of the vascular space in the wall of which they are located. Although these nodules may narrow the lumen somewhat, their bulk usually projects into the adventitial tissue (fig 3). Some nodules are of spindle shape, others are sharply demarcated from the adjacent vascular wall, and may even follow a polypoid outline, again, in others there is a gentle tapering off to the adjacent portion of the wall. They may be multiple. In longitudinally sectioned vascular spaces two of these nodules may face each other, suggesting the presence of a partial or complete ring of the tissue of which they are composed (figs 4 and 9).

Although these nodules do not seem to be present in every capillary hemangioma, they are not an uncommon finding. While in some tumors one may search for a long time, and often in vain, others display a considerable number of various size, shape and cellular pattern. In the following paragraphs their development will be traced from small epithelioid "beads" in the walls of vascular units of capillary size until they reach the respectable size which is seen in some of the larger units. This development appears to be essentially a



Fig 3—A mural nodule of a larger vascular unit of a capillary hemangioma. It narrows the lumen somewhat, but its bulk projects into the adventitial tissue. A few deeply staining myofibrils are seen in several portions of the wall of the unit. On reaching the nodule this scanty muscle layer appears to split up. It is all but lost in the nodular bulk except for a few isolated fragments. Mallory's phosphotungstic acid-hematoxylin,  $\times 150$ .

Fig 4—Two "facing" mural nodules of a larger unit of a capillary hemangioma. Hematoxylin-eosin,  $\times 120$ .

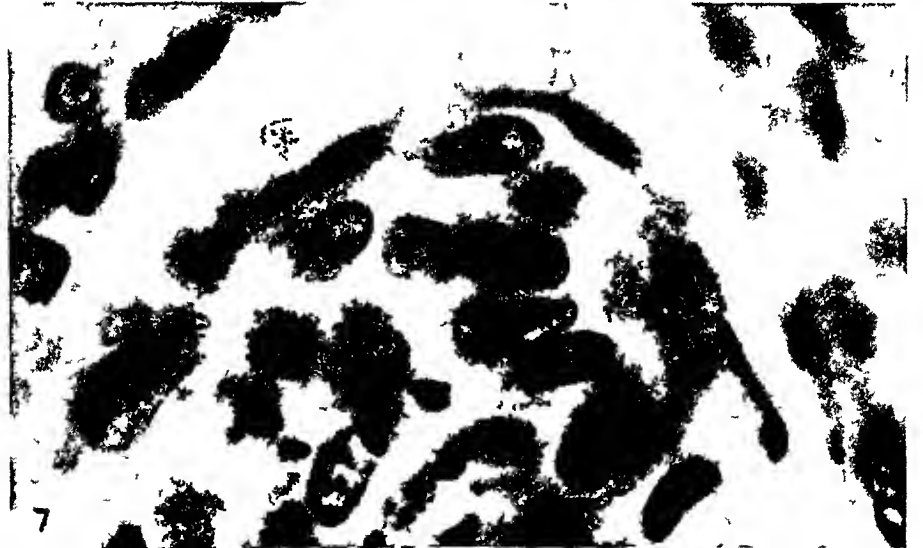


Fig 5—Early beading of the wall of a vascular unit of a capillary hemangioma. The endothelium appears pushed into the lumen by the mural cells. There is slight vacuolation of the cytoplasm of the mural cells, and a small amount of nuclear debris is scattered about. Hematoxylin-eosin,  $\times 1,260$

Fig 6—Early beading of the wall of a vascular unit. The cytoplasmic vacuolation is more conspicuous, and there is an increase in the amount of nuclear debris. The endothelium appears to be lifted from the mural cells. The epithelioid character of the mural cells is lost. In the right lower corner an isolated mural cell is located adjacent to, parallel to, and at the same level as, an endothelial cell of a vascular unit. Hematoxylin-eosin,  $\times 1,260$

Fig 7—Small mural nodule of a vascular unit. The lifting of the endothelium is more conspicuous, and there is considerable increase of the nuclear debris. Hematoxylin-eosin,  $\times 1,350$

regressive process, of which the fully developed nodule represents the end stage. Therefore it is understandable that mural epithelioid cells and mural nodules are only rarely seen in the same tumor.

It will be remembered that in the walls of the small units the differentiation of endothelial and mural cells is uneven. Small clusters of clear epithelioid cells are seen next to undifferentiated, fibroblast-like portions. It will be shown, in a series of transitional pictures, how a small segment of wall with mural-endothelial differentiation may give rise to mural nodules.

Figure 5 shows early beading of the wall of a unit in which there has been good differentiation of endothelial and mural elements, with several epithelioid cells being present in the wall. The "bead" consists of a small group of swollen, clear cells, bulging into the lumen and covered with thin, stretched-out endothelium. Within the body of the "bead" are several dark, basophilic coarse irregular granules, a common feature of the early and moderately advanced stages of the formation of mural nodules. In these stages it is seen so frequently that it lends itself well as a landmark if one is searching for structures of this nature. There is no appreciable number of intercellular structures.

Figure 6 shows a more advanced nodule, but similar in size and shape to that of figure 5. Several features are worth noting. The endothelium appears lifted off the mural cells, a detachment which is frequently observed, in paraffin sections, in the early stages. There also is considerable vacuolation of the cytoplasm of the mural cells. Occasionally one may see a round refractile body in such a vacuole, suggestive of a red blood cell, or a coagulum of liquefied plasma. The basophilic clumps and granules are considerably increased in number. Figure 6 also shows loss of distinct cell boundaries and elongation of the mural nuclei, which thereby assume the appearance of fibroblasts. A few isolated reticulum fibrils are seen in nodules of this kind, occasionally in continuity with the peripheral reticulum.

A similar stage is seen in figure 7. The lesion is larger and more cellular. The vacuolation of mural cells, with lifting of the endothelium, is pronounced, nuclear debris is abundant. Intercellular structures are not yet conspicuous, and there is no reaction of the stromal reticulum. In figure 8 the vascular unit is of larger caliber. The nuclei are spaced farther apart, and many intercellular fibrillar structures are seen with the hematoxylin-eosin stain. At this stage argyrophilic fibrils are usually scanty except in the periphery. In figure 8 a wavy bundle of stromal reticulum fibrils is seen hugging the mural periphery, some of these appear to penetrate the outer portion of the mural body.

Figure 9 shows an example of facing nodules, conceivably representing a mural ring. Both nodules are essentially similar, with fibroblastic mural cells and moderate fibrosis, particularly in the periphery. There is some nuclear debris and some cytoplasmic vacuolation. On the left side, in the nodular periphery, there is a conspicuous adventitial fibroblast adjacent to a large number of reticulum fibrils. The fibrosis also is more pronounced on the left than on the right.

The fibrosis is even more pronounced in figure 10. The peripheral half consists of fibrous tissue of low cellularity, while a considerable number of fibroblastic nuclei are still seen in the inner portions. A few argyrophilic fibrils are seen occasionally in the inner areas of nodules of this size, but they do not appear to be continuous with those seen in the nodular periphery. Figure 11 shows the dense character of the intercellular substance, particularly in the periphery, where in places dense, wavy collagenous bundles are seen.

Finally, in figures 3, 4 and 7 the difference between the center and the periphery of the nodule is absent. The lesions consist of fibrous tissue of low cellularity. The nuclei frequently are arranged parallel to the intimal surfaces, except at the two poles of the spindle, toward which the nuclei can be seen to converge, thereby accentuating the spindle shape of the structure.

Nerve fibrils cannot be demonstrated in connection with the epithelioid cells and the mural nodules by using Mallory's aniline blue stain and del Río Hortega's silver carbonate stain. It is also exceedingly rare to find muscle elements in the nodular substance, and then one finds them only if the unit in which the nodule is located is of considerable size. This is in line with the observation that muscular structures in general are absent or deficient in angiomas.<sup>1</sup>

Figure 3 depicts a phosphotungstic acid-hematoxylin stain of a vascular unit in which a more or less complete ring of smooth muscle tissue, though thin and varying in thickness, is seen in the wall of the unit, in its non-nodular portion. Here the myofibrils are located roughly between the inner and the outer half of the wall. On approaching the nodule the muscle layer is seen to split up, being traced only with difficulty at the nodular poles. The ends of a few fibrils show a gentle outward curving before their trace is lost in the nodular bulk. A few isolated fragments of myofibrils are still to be seen in the middle and outer portions of the nodule. The sum total of the latter fragments may be equal to the number seen in a corresponding segment of the non-nodular portions. It hardly exceeds that

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<sup>1</sup> Ewing, J. *Neoplastic Diseases*, ed 4, Philadelphia, W. B. Saunders Company, 1942, p. 251.



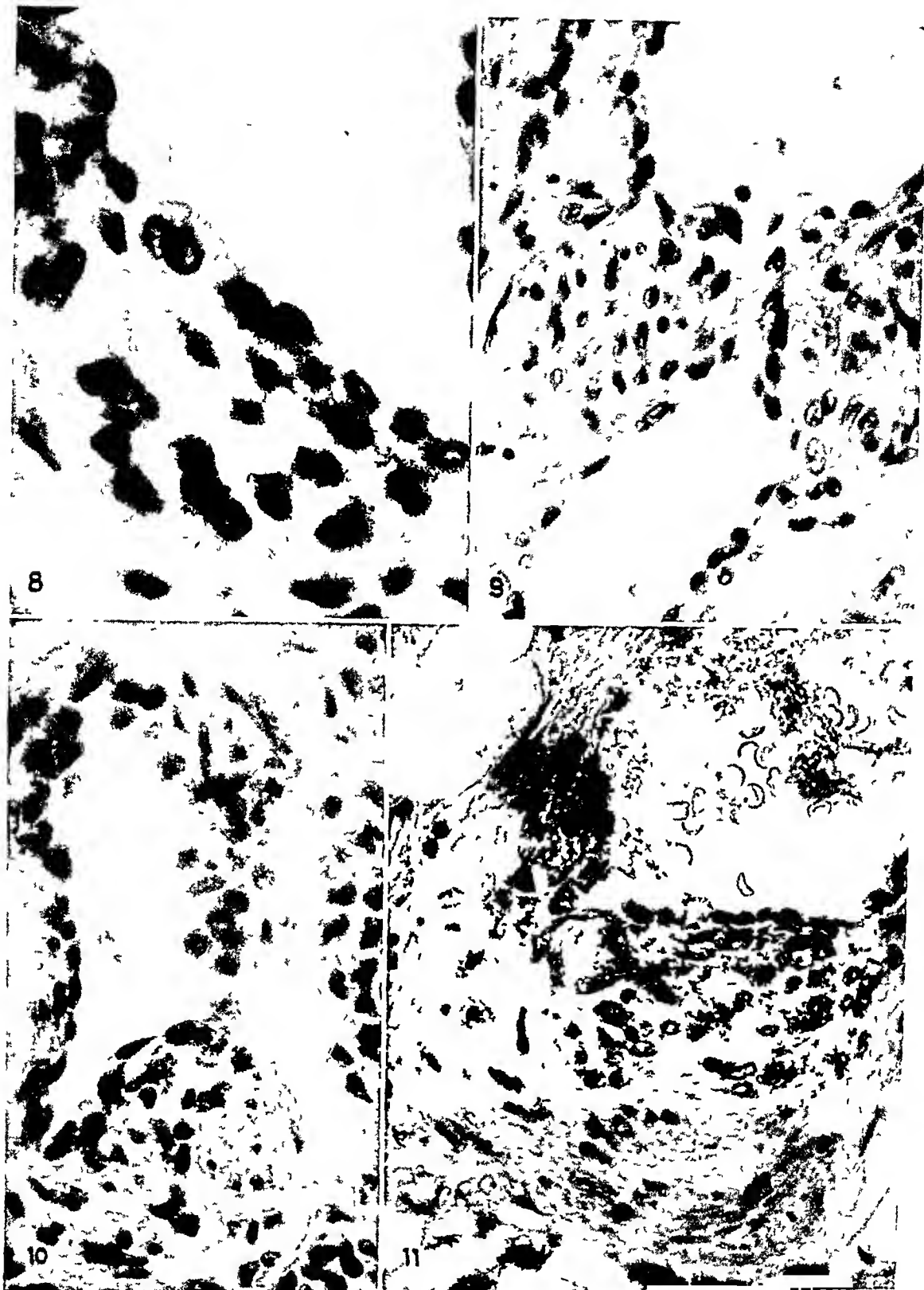


Fig 8—The mural nodule is larger and more fibrous than that seen in figure 7. There is a wavy bundle of collagen hugging the periphery of the nodule at the bottom of the photomicrograph. Hematoxylin-eosin,  $\times 681$ .

Fig 9—Facing nodules, conceivably representing a mural ring. The peripheral fibrosis is more pronounced on the left side. There also is a conspicuous adventitial fibroblast adjacent to the reticulum fibrils in the periphery of the nodule. Hematoxylin-eosin,  $\times 510.5$ .

Fig 10—A mural nodule in a unit of medium caliber. The difference of cellularity of the inner and the outer portion is obvious. Hematoxylin-eosin,  $\times 510.5$ .

Fig 11—A mural nodule showing a higher degree of fibrosis and dense collagenous tissue in the periphery. Hematoxylin-eosin,  $\times 681$ .

## COMMENT

Essentially two questions pose themselves to the observer of the structures described. These questions are closely related to each other and are not without bearing on the problem of the nature of angiomas: (1) What is the nature and origin of the epithelioid cells seen in the walls of vascular units of the capillary hemangioma? (2) What is the nature of the mural nodules seen in some of the larger units of this tumor?

To the casual observer there appears to be a resemblance between the mural epithelioid cells and the "epithelioid" cells of the glomus and its tumors,<sup>2</sup> the "epithelioid" cells of other arteriovenous anastomoses<sup>3</sup> and the juxtaglomerular apparatus. One may therefore speculate whether there is not also a functional resemblance, and whether the epithelioid cells in capillary hemangioma do not represent afibrillar muscle cells, conceivably with a motor function affecting the blood flow within the angiomatous bed. This theory may receive support from the fact that configurations resembling arteriovenous anastomoses are occasionally seen in angiomas and that arteriovenous anastomoses are frequently found in the immediate vicinity of the corpora cavernosa of the penis and the clitoris, organs whose structure resembles cavernous hemangioma. Similarly, the mural nodules, on first glance, resemble cushions of smooth muscle cells. One may therefore also speculate whether these nodules might not have a similar significance.

The observations described in this note disclose no evidence that epithelioid mural cells are, or develop into, elements of muscle cell character. Similarly, the number of contractile elements in the mural nodules is so negligible that any functional significance can be safely denied.

At this point it is worth while to remember that the smooth muscle cells of blood vessels are of local origin<sup>4</sup> and are not derivatives of the angioblastic mesenchyme. Although this fact is not material in answering the questions posed in a foregoing paragraph—as the epithelioid cells apparently are not myoblastic elements, and the mural nodules are not cushions of smooth muscle cells—it makes one wonder whether the epithelioid cells are of local or of angioblastic origin. Off hand this question appears purely academic. Neither can morphologic studies such as the preceding one answer it. But the evidence can be evaluated.

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2 Bailey, O. T. The Cutaneous Glomus and Its Tumors—Glomangiomas, *Am J Path* **11** 915-935, 1935

3 Schumacher, S. Ueber die Bedeutung der arteriovenösen Anastomosen und der epitheloiden Muskelzellen (Quellzellen), *Ztschr f mikr-anat Forsch* **43** 107-130, 1938

4 Maximow, A. A., and Bloom, W. A Textbook of Histology, ed 4, Philadelphia, W. B. Saunders Company, 1943, p 241

This evidence points toward an angioblastic origin of the epithelioid cells. True, pictures are found suggestive of an induction of the adventitial stroma overlying the endothelium, to form epithelioid cells. An example of this is seen in figure 6, in the right lower corner, where an isolated mural (or stromal) cell with oblong nucleus and clear cytoplasm is found adjacent to, parallel to, and at the same level as, an endothelial cell of the vascular unit. A similar picture is seen in the left lower corner of figure 5. It also is easy to construe transitional cells between stromal and mural epithelioid elements. An example of this is seen in figure 10, midway between the upper and the lower pole of the vascular unit, on its right side, where there is a large epithelioid cell, with indented nucleus and clear cytoplasm, seemingly free in the stroma, but favoring the unit in the center of the photograph. The over-all evidence, however, favors an angioblastic origin.

Most epithelioid cells appear to develop from tumor cells which have not yet differentiated into endothelial and mural elements. Also there is evidence that many mural cells have preserved certain vasoformative properties. This is suggested by the frequent vacuolation of their cytoplasm and the basophilic coarse debris, both features seen in the early nodules and described in the foregoing pages in detail.

Intracellular vacuolation, with vacuolar fusion to form capillary spaces, is seen occasionally in undifferentiated portions of angiomas and can be interpreted as a vasoformative attempt similar to the process of luminization of angioblastic tissue in the early embryo<sup>5</sup>. This argument gains weight from the occasional presence of refractile or non-refractile bodies in these vacuoles which may be red blood cells or coagulums of liquefied cytoplasm.

The basophilic clumps and granules represent nuclear debris. Their presence denotes cell death. Cell death, with the appearance of pyknotic and fragmented nuclei, is also seen during the process of luminization of solid angioblastic tissue<sup>5</sup>.

The nature of the mural nodule is more difficult to assess. There is some evidence that it is derived from two sources, the mural cells and the local stroma. This is suggested by the observation that frequently it is divided into an outer more fibrous and an inner more cellular portion, that the stromal reticulum fibrils apparently extend into the periphery in the early stage of its development and that certain stromal reticulum cells conspicuously hug the nodular periphery in periods of what appears to be increased deposition of intercellular fibrillar matter.

Also, if one accepts the evidence that the mural epithelioid cells play a role in the early stage of formation of the nodule as outlined

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5 Sabin, F. R. Studies on the Origin of Blood Vessels and of Red Blood Corpuscles as Seen in the Living Blastoderm of Chicks During the Second Day of Incubation, *Contrib. Embryol.* 9: 213-263, 1920.

in this note, and if one accepts the evidence that the nodule is of an angioblastic, nonmyoblastic nature, one is forced to acknowledge that at some late period of the nodule formation the local stroma plays a part. How else could one explain the presence of myofibrils, scanty and sporadic as they may be? Similarly the local stroma must have contributed to the formation of the wall of the larger vascular units, particularly those which display contractile elements.

The development of mural nodules can then be interpreted as a replacing of foci of angioblastic tissue with predominantly fibrous elements. It is therefore basically a regressive process, although in the early stages the tissue is quite cellular and mitotic figures abound. It therefore can be compared with other regressive changes which are observed in angiomas, one of the best known being that of sclerosis as described by Gross and Wolbach.<sup>6</sup> Their common feature is the connective tissue replacement of vascular tissue.

It is of interest that structures similar to mural nodules are seen in the blood vessels of the embryo.<sup>7</sup> When a fetal vessel undergoes regression, it is not uncommon for its wall to show similar mesenchymatous cushions where the vessel joins a nonregressing vessel. If this process is accentuated, these cushions may extend into and around the wall of the nonregressing vessel—an event of possible significance in the development of certain vascular malformations.

The mural nodules of the capillary hemangioma can then be considered the result of a focal regressive change of angioblastic tissue, suggestive of a recapitulation of the normal developmental sequence observed in the fetal vascular tree.

#### SUMMARY

Several observations concerning the nonendothelial tumor cells found in capillary hemangioma are reported.

Fibrous nodules occurring in the walls of the vascular units of capillary hemangioma are described.

The development of these nodules is traced from the nonendothelial tumor cells.

The nature of the nonendothelial cells and of the mural nodules is discussed, with special consideration of their relation to contractile elements.

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6 Gross, R. E., and Wolbach, S. B. Sclerosing Hemangiomas, *Am J Path* 19 533-551, 1943.

7 Bremer, J. L. Personal communication to the author.

# ASYMPTOMATIC RETENTION OF PANCREATIC SECRETION

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IN THE course of routine histologic examinations of necropsy tissues we have observed from time to time retention of eosinophilic secretion in the acini of pancreases removed from patients in whom during life there was no clinical evidence of cystic fibrosis of the pancreas. The retention was, however, occasionally found concomitantly with staphylococcic bronchitis and bronchiectasis, an integral feature of cystic disease of the pancreas. This report deals with the cases in which apparently asymptomatic pancreatic retention was observed in the necropsy material in this hospital in a four year period (1944-1947).

Few reports relating to acinous retention of pancreatic secretion other than that occurring in cystic fibrosis of the pancreas have been found in the literature. In 1947 Baggenstoss<sup>1</sup> described inspissation of secretion in the acini with some flattening of the lining epithelial cells in patients dying of uremia. This retention of secretory material was found in 33 of 85 cases in which glomerulonephritis terminated in uremia and in 52 cases in which uremia resulted from miscellaneous causes. Furthermore, a similar change was found in 20 per cent of a control series of 200 cases. No definite contributory factor was demonstrated, but the most common cause of death was intestinal obstruction. Mallory<sup>2</sup> in a discussion of that paper stated that in 10 per cent of the autopsy material seen in his laboratory in Italy a similar pancreatic lesion was found. It was most pronounced in patients dying of typhus fever. Gilman<sup>3</sup> observed a similar pancreatic reaction with changes in the liver in rats experimentally subjected to nutritional deficiencies.<sup>3a</sup>

## MATERIAL AND FINDINGS

Critical examination was made of the pancreatic tissue at all routine necropsies during the years 1944 to 1947, inclusive. During this period 10 cases (39 per cent) of definite cystic fibrosis of the pancreas occurred. Sections were fixed in Zenker's solution and in 4 per cent formaldehyde solution and were

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From the Pathologic Laboratories of the University of Pittsburgh and the Children's Hospital of Pittsburgh

- 1 Baggenstoss, A. H. Am J Path 23 908, 1947
- 2 Mallory, T. B. Am J Path 23 908, 1947
- 3 Gilman, T. Am J Path 23 908, 1947

stained with hematoxylin and eosin. The 256 necropsies included in this study disclosed 35 pancreases which showed varying degrees of pancreatic secretory retention not related to cystic fibrosis. This number comprised only those pancreases in which the retained secretion was readily discernible. Several in which the degree of retention or the amount of tissue involved was minimal were excluded. The ages of the patients varied from 10 days to 16 years.

No gross abnormality of the pancreas was described in any of the necropsy protocols.

Microscopically, the retained secretion varied in amount and distribution. The number of acini involved varied between 10 and 25 per cent. In more than one half of the affected pancreases not only acini but intralobular and interlobular ducts as well were involved, in the remaining glands retention was

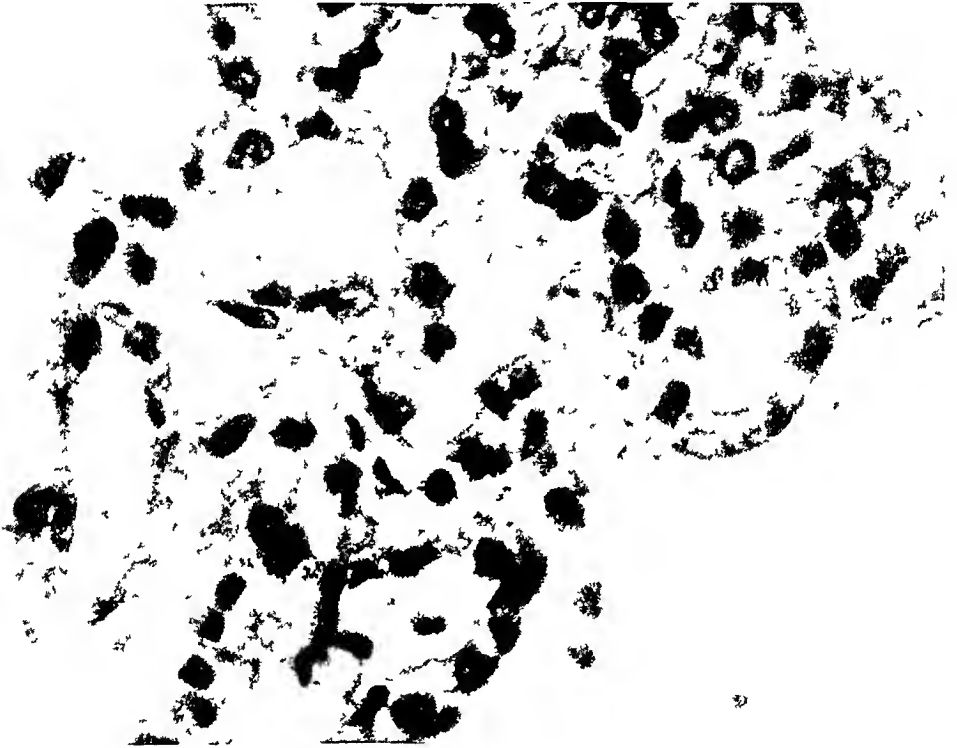


Fig 1—Dilated acini with retained secretion and flattening of the bordering epithelium  $\times 725$

limited almost entirely to the acini and ductules, or to the ductules and ducts, as indicated in the table. The inspissated material most frequently occurred in the central areas of the lobules, and different lobules varied considerably in the degree of involvement.

In a well developed lesion, about one quarter of the tissue was involved. The acini exhibited varying dilatation and were irregularly distributed. Under high power, the inspissated eosinophilic material appeared homogeneous or finely granular, and frequently the encircling epithelial cells appeared flattened. Occasionally the epithelium showed degeneration with many residual pyknotic nuclei, and in a few instances it had almost entirely disappeared. The surrounding acini, which were free of retained secretion, contained little or no zymogen. The involved ductules showed varying degrees of dilatation, and the retained

# Cases of Pancreatic Retention

| Autopsy | Age     | Sex | Site of Pancreatic Retention |          |       | Pathologic Change in Lung |                    |            |                        | Culture of Material from Lung  | Anatomic Diagnosis   | Comment                           |
|---------|---------|-----|------------------------------|----------|-------|---------------------------|--------------------|------------|------------------------|--------------------------------|--|-----------------------------------|
|         |         |     | Acm                          | Ductules | Ducts | Acute Bronchitis          | Bronchitic Abscess | Bronchiole | Interstitial Pneumonia |                                |  |                                   |
| 1944    |         |     |                              |          |       |                           |                    |            |                        |                                |  |                                   |
| 4       | 3 yr    | F   | +                            | +        | +     |                           |                    |            | +                      | Staph albus                    | Diabetes mellitus, pulmonary emphysema, atelectasis                                      |                                   |
| 8       | 11 yr   | F   | +                            | +        | +     | +                         |                    |            |                        | Pneumococcus VI Staph albus    | Bronchopneumonia   | Interlobular fibrosis of pancreas |
| 11      | 2 mo    | M   | +                            | +        | +     | +                         | +                  |            |                        | Staph aureus                   | Bronchopneumonia   | Neutrophils in dilated acini      |
| 26      | 2 mo    | M   | +                            | +        | +     | +                         | +                  |            |                        | Negative                       | Biliary cirrhosis  |                                   |
| 41      | 13 mo   | F   | +                            | +        | —     | +                         | +                  |            |                        | Staph albus and aureus         | Hydronephrosis   |                                   |
| 3       | 1 mo    | M   | —                            | +        | +     | +                         | +                  |            |                        | Staph albus                    | Bronchopneumonia, congenital heart disease (aortic stenosis, coarctation of aortic arch) |                                   |
| 9       | 15 mo   | F   | —                            | +        | +     |                           |                    |            | +                      | Staph albus                    | Laryngotracheal bronchitis   |                                   |
| 1945    |         |     |                              |          |       |                           |                    |            |                        |                                |  |                                   |
| 2       | 6 mo    | F   | ++?                          | +        | +     |                           |                    |            | +                      | Staph albus                    | Congenital heart disease (interauricular septal defect), primary atypical pneumonia      | Degeneration of pancreatic acini  |
| 22      | 6 yr    | M   | +                            | +        | +     |                           |                    |            | +                      | Ps aeruginosa                  | Biliary cirrhosis  |                                   |
| 38      | 14 days | M   | ++?                          | +        | +     | +                         | +                  |            |                        | Alcaligenes faecalis           | Primary atypical pneumonia   |                                   |
| 37      | 10 mo   | M   | +                            | +        | +     |                           |                    |            | +                      | Negative                       | Hydrocephalus  |                                   |
| 41      | 2 yr    | F   | +                            | +        | +     | +                         | +                  |            |                        | Staph aureus                   | Multiple abscesses of lungs  |                                   |
| 6       | 18 mo   | F   | —                            | +        | +     |                           |                    |            | +                      | Negative                       | Primary atypical pneumonia   |                                   |
| 1946    |         |     |                              |          |       |                           |                    |            |                        |                                |  |                                   |
| 23      | 11 yr   | M   | +                            | +        | +     |                           |                    |            | +                      | Staph aureus                   | Lymphosarcoma  | Tumor metastases in pancreas      |
| 32      | 3 mo    | M   | +                            | +        | +     |                           |                    |            | +                      | Staph albus and Strep viridans | Primary atypical pneumonia   |                                   |
| 35      | 2 yr    | M   | +                            | +        | +     | +                         |                    |            |                        | Strep hemolyticus              | Lymphatic leukemia, bronchitis   |                                   |
| 11      | 16 yr   | F   | +                            | +        | —     |                           |                    |            | +                      | Strep viridans                 | Rheumatic pancarditis  |                                   |

| Autopsy | Age     | Sex | Site of Pancreatic Retention |       | Pathologic Change in Lung |             |              |   | Culture of Material from Lung | Anatomic Diagnosis  | Comment  |
|---------|---------|-----|------------------------------|-------|---------------------------|-------------|--------------|---|-------------------------------|---|--|
|         |         |     | Acm                          | Ducts | Acute Bronchitis          | Bronchioles | Interstitium |   |                               |   |  |
| 12      | 1 mo    | M   | +                            | +     | —                         | —           | —            | — | Ps aeruginosa                 | Atelectasis of lung, acute enteritis                                  | Diarrhea of newborn  |
| 50      | 2 mo    | F   | +                            | +     | —                         | —           | +            | + | Staph albus                   | Cholangitis, primary atypical pneumonia                               |  |
| 62      | 10 yr   | F   | +                            | +     | —                         | —           | —            | + | Staph albus                   | Lymphosarcoma   |  |
| 7       | 4 mo    | F   | —                            | +     | +                         | +           | +            | + | Pneumococcus *                | Tracheoesophageal fistula   |  |
| 45      | 10 days | M   | —                            | +     | +                         | +           | +            | + | Strep hemolyticus             | Atresia of duodenum   |  |
| 61      | 13 yr   | F   | —                            | +     | +                         | +           | +            | + | Not taken                     | Osteogenic sarcoma  |  |
| 1947    |         |     |                              |       |                           |             |              |   |                               |   |  |
| 8       | 1 mo    | F   | +                            | +     | +                         | +           | +            | + | Staph aureus, pneumococcus *  | Primary atypical pneumonia, acute enteritis                           | Diarrhea of newborn  |
| 13      | 5 mo    | M   | +                            | +     | +                         | +           | +            | + | Staph aureus                  | Purulent tracheobronchitis  |  |
| 18      | 6 mo    | M   | +                            | +     | +                         | +           | +            | + | Negative                      | Subdural hematoma   |  |
| 50      | 5 days  | F   | +                            | +     | +                         | +           | +            | + | Staph aureus                  | Acute enteritis, subdural hematoma                                    | Diarrhea of newborn, in filtration of interlobular connective tissue and ducts of pancreas |
| 37      | 13 yr   | M   | +                            | +     | +                         | +           | +            | + | Negative                      | Miliary tuberculosis  |  |
| 76      | 1 mo    | M   | +                            | +     | +                         | +           | +            | + | Strep viridans                | Primary atypical pneumonia, pyloric stenosis                          |  |
| 88      | 5 mo    | F   | +                            | +     | +                         | +           | +            | + | Negative                      | Encephalitis  |  |
| 35      | 14 yr   | M   | +                            | +     | +                         | +           | +            | + | Negative                      | Rheumatic pancreatitis  |  |
| 40      | 2 mo    | F   | +                            | +     | +                         | +           | +            | + | Staph albus                   | Primary atypical pneumonia  |  |
| 19      | 2 mo    | M   | —                            | +     | +                         | +           | +            | + | Staph albus, pneumococcus *   | Congenital heart disease (patent ductus arteriosus and foramen ovale) |  |
| 21      | 3 mo    | M   | —                            | +     | +                         | +           | +            | + | Pneumococcus *                | Internal hydrocephalus  |  |
| 83      | 4 days  | F   | —                            | +     | +                         | +           | +            | + | Negative                      | Primary atypical pneumonia  |  |

\* Type not determined



eosinophilic material was vacuolated along the outer edges. In many of the dilated ductules there was flattening of the epithelial lining with pyknotic nuclei (fig 1). Where the process was less pronounced, the acini contained only small amounts of secretion, the dilatation was minimal and there was little change in the epithelial lining (fig 2A). In a few cases, especially in those

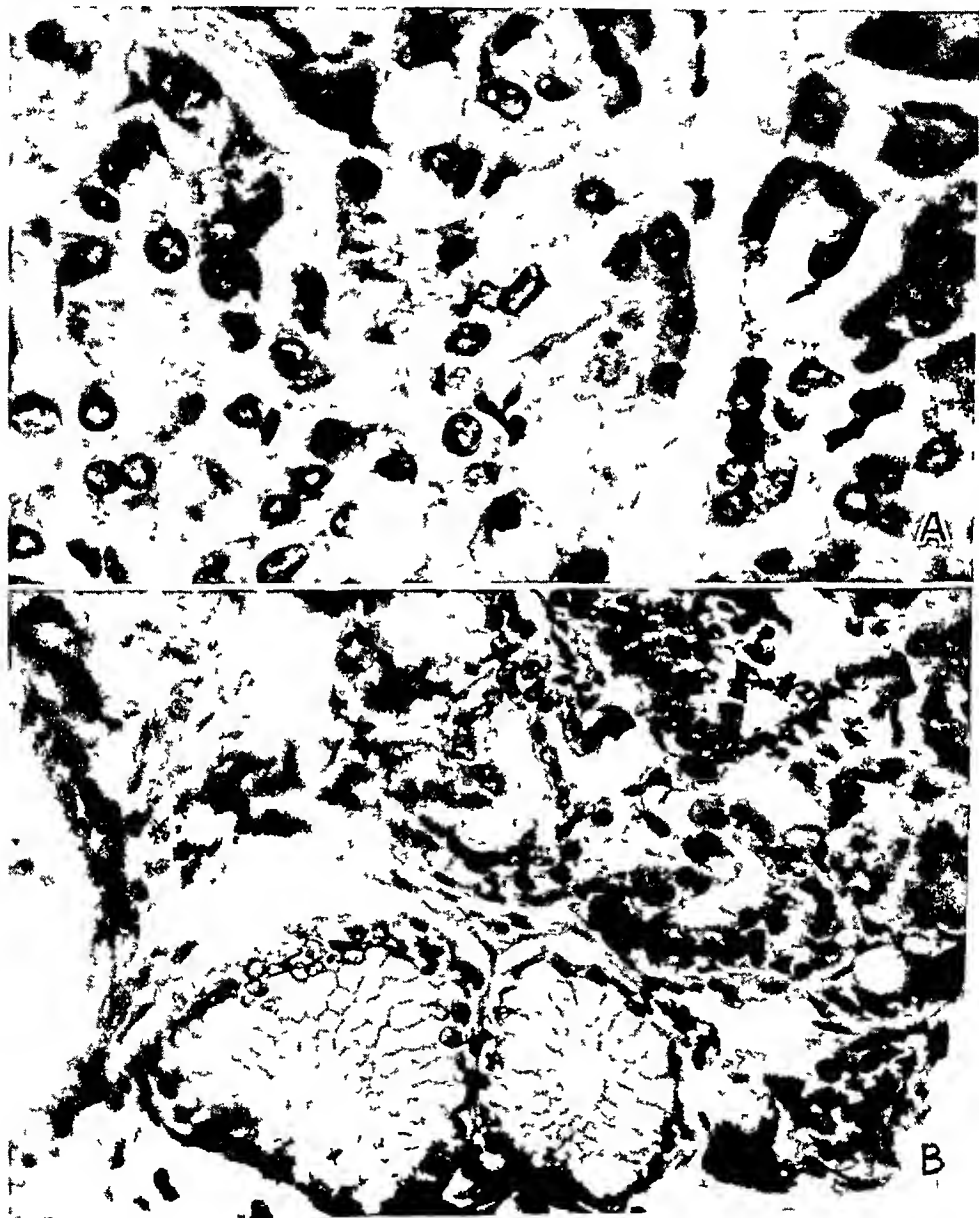


Fig. 2—*A*, moderate dilatation of acini and minimal retention of secretion. The epithelium of these acini is fairly normal  $\times 725$

*B*, dilated acini and also two acini showing extreme vacuolation of cells with nuclei pushed to the periphery  $\times 650$

in which the cause of death was rheumatic pancarditis, vacuolation of the cytoplasm was observed in some of the acinous epithelial cells. This vacuolation began as small vesicles along the luminal border of the acinous cells

As the process advanced, the entire cytoplasm became converted into clear vacuoles, with the nucleus pushed to the periphery (fig 2B)

In 2 cases (no 26 in 1944 and no 19 in 1947), in which the anatomic diagnoses were biliary cirrhosis and congenital heart disease, respectively, the secretion filling the dilated acini also contained neutrophils. Interlobular fibrosis was a concomitant finding in 2 instances (nos 13 and 50 in 1947) diagnosed anatomically as instances of cholangitis and acute tracheobronchitis, respectively. Focal lymphocytosis of the interlobular connective tissue occurred in case 22 (1945), a case of biliary cirrhosis, and in case 50 (1947), a case in which death followed subdural hemorrhage and diarrhea.

The associated pulmonary lesions were interesting. In 17 of the 35 cases there was an accompanying acute bronchitis, occasionally of abscess proportions with bronchiectasis and patchy bronchopneumonia. Cultures of the lungs revealed a staphylococcus to be the predominating organism in 17 instances. However, only in 7 of these was this *Staphylococcus aureus*. The lung in 9 cases showed an intestinal involvement as in primary atypical pneumonia of undetermined origin.

#### COMMENT

The cause of death in the patients in whom the retained pancreatic secretion occurred varied so widely that it has been impossible to ascribe definitely any factor as a causative agent of the retention. The most pronounced lesions were found in 2 cases of diarrhea of the newborn. Baggenstoss, Power and Grindlay<sup>4</sup> likewise observed a high incidence of this lesion in diseases of the intestinal tract. The drastic changes in metabolism in the 2 diarrheic infants, as well as in the terminal state in some of the other patients in our series, suggests that the basis for the perverted secretion may lie in a disturbance of metabolism interfering with proper reparative synthesis of the pancreatic secretion. The zymogen in normal pancreatic cells is apparently depleted and renewed about every twenty-four hours. The synthesis of a secretory enzymatic product capable of splitting protein, fats and carbohydrates is conceivably complex, and the final product might readily be altered in composition by deficiencies or changes in the precursors necessary for its formation. The decrease of trypsin in the pancreatic juice in cystic fibrosis and the diminution of amylase in celiac disease recently shown by Andersen<sup>5</sup> are evidence of qualitative chemical alterations which may obtain in pancreatic secretion. The first condition is irreversible and fatal, the second responds to treatment. Andersen further suggested that there is some minor deficiency in the digestion of protein in celiac disease. It seems plausible, therefore, to anticipate that the drastic changes in metabolism which occur in fatal uremia, diarrhea of the newborn or pyloric stenosis may be reflected in the anlage necessary for the synthesis of the pancreatic secretory product. A second hypothesis recently suggested by Baggenstoss, Power and Grindlay<sup>4</sup> for the origin of cystic

4 Baggenstoss, A. H., Power, M. H., and Grindlay, J. H. *Am J Path* 24: 692, 1948.

5 Andersen, D. *Am J Dis Child* 63: 643, 1942.

disease of the pancreas might likewise be applied to the etiology of benign retention of pancreatic secretion. According to this theory, either a deficiency or an inhibition of secretin, the hormone which is responsible for the more watery character of the secretory product flowing into the acini, increases the viscosity of the pancreatic juice. This increased viscosity could lead to retention and inspissation in the acini and ducts.

The fact that the pancreases showing marked acinous retention associated with epithelial degeneration are identical with those observed in early cases of pancreatic cystic disease, as has been illustrated by Farber's<sup>6</sup> studies, suggests that knowledge of the mechanism of the benign retention may yield an approach to the solution of the genesis of cystic fibrosis of the pancreas.

#### SUMMARY

During the years 1944 to 1947, in 35 (13.7 per cent) of the 256 autopsies performed pancreatic tissues were removed which showed microscopically a retention of acinous secretion not related to cystic disease of the pancreas and not associated with clinical symptoms. The alteration of the secretion was apparently due to some metabolic fault reflected in the synthesis of pancreatic enzyme.<sup>7</sup>

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<sup>6</sup> Farber, S. *Arch Path* **37** 238, 1944.

<sup>7</sup> In the interim between the acceptance and publication of this article there appeared another article by A. H. Baggenstoss (*Am J Path* **24** 1003, 1948) in which similar retention of pancreatic secretion was described in pancreases from adults.

## A PROTEIN COMPONENT OF THE GOLGI APPARATUS

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**S**TUDIES on the composition of the Golgi apparatus are marked by an emphasis on the lipid components of this structure. This is readily understandable since some lipids are rather readily demonstrated and since peculiarly specific conditions utilizing quasilipid methods often are required to visualize the Golgi apparatus in permanent preparations. Studies on possible protein components are so equivocal that Baker,<sup>1</sup> in 1944, was moved to write

The suggestion that the Golgi element contains protein does not rest on strictly histochemical evidence. It is perhaps unlikely that any part of the cell is totally lacking in proteins, but histochemical tests for them have not been shown to give positive reactions in the Golgi element.

The chief evidence bearing on this subject is the stainability of the Golgi apparatus when Weigert's resorcinofuchsin, the iron-hematoxylin technic, aniline blue and nigrosin are used.

This paper is concerned with a description of what appears to be a carbohydrate-containing protein in the Golgi apparatus of several types of cells, primarily the columnar cells of the mucosa of the duodenum of the rabbit and the guinea pig. An attempt was made to characterize this substance by the use of solvents and enzyme preparations.

### MATERIAL AND METHODS

Tissues were prepared by freezing and drying and were then embedded in paraffin. Sections were deparaffinized and were either coagulated with alcohol directly or after being treated with a reagent. They were then stained for polysaccharides according to the method suggested by Hotchkiss.<sup>2</sup> This procedure involves the oxidizing to aldehydes of certain alcohol groups of carbohydrates bound to tissue substance and visualizing the compound so formed as a red color by the use of the Feulgen reagent. When sections are coagulated with alcohol, the Golgi apparatus of the columnar duodenal cell appears clearly as a delicate

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This investigation was aided in part by a grant from the American Cancer Society recommended by the Committee on Growth, National Research Council.

1 Baker, J. R. *Quart J Micr Sc* 85 1, 1945.

2 Hotchkiss, R. D. *Arch Biochem* 16 131, 1948.

pink or red network differentiated from the just perceptibly pink or colorless supranuclear protoplasm. Only when a reagent results in marked diminution or complete absence of stainability of the Golgi apparatus is it considered that the reagent has taken part in removal of the reactive components.

It may be helpful at this point to supplement the discussion of Hotchkiss on the specificity of the periodic acid—leukofuchsin method under these conditions. Carbohydrates may occur free in sections of frozen-dried material or combined in the forms of a polysaccharide (glycogen), certain lipids (considered in detail in the following pages), possibly lipoproteins and glycoproteins. Freely diffusible reactive components pass into solution from the section in the various steps of the staining procedure. Glycogen may be removed by the use of saliva after protein coagulation. The following lipids (free) have been found to give a purple-red addition product with the Feulgen reagent after being oxidized with periodic acid: kerasin, phrenosin, phosphatidyl ethanolamine, acetal phosphatide, inositol phosphatide, lecithin and ganglioside.<sup>3</sup> All of the free lipids may be removed by prolonged extraction with a hot mixture of equal parts of chloroform and methanol. However, it is possible that some or all of these lipids may be combined as lipoproteins. The glycolipid-protein complex recently described by Folch and Uzman<sup>4</sup> might be expected to be extracted if the section is treated with hot methanol-chloroform for sixteen hours. It is possible that other lipoprotein complexes may not be extracted by the procedure employed and would remain in the section with enough appropriate reactive groups retained to give a red stain with the periodic acid—leukofuchsin reaction. In addition to these compounds, the only remaining substances which would give a positive red color would then be carbohydrate-protein complexes. The distinction between lipoproteins and glycoproteins may be clarified by further studies of the extractabilities of stainable components following the application of chemical reagents and enzyme preparations. Thus, if the stainable component shows an isoelectric point and other properties corresponding to those of a chemically isolated and known glycoprotein, then the stainable component may be identified rather clearly as such a compound. Again, if a stainable component is extracted after the application of hyaluronidase, it may reasonably be assumed to be a glycoprotein. However, in other instances, where less is known of the chemical nature of purified extracts or of the properties of the stainable cell components in sections, it may become difficult or impossible to distinguish clearly between, or identify, reactive groups as component parts of lipoproteins or of glycoproteins.

The details of the procedure used are as follows. Five normal adult rabbits and 6 normal adult guinea pigs were killed by a blow on the head. Very small snips of the cephalic portion of the duodenal mucosa were removed and immersed in isopentane at approximately  $-160^{\circ}\text{C}$ . After freezing they were dried in a vacuum at about  $-30^{\circ}\text{C}$ , infiltrated with paraffin (melting point,  $56$  to  $58^{\circ}\text{C}$ ) during fifteen minutes, and embedded. Sections 6 microns thick were cut and mounted with slight pressure on an albuminized slide. The section was warmed again until the paraffin was just melted and then was flattened with slight pressure. The standard of reference was prepared by removing the paraffin with xylene or benzine and coagulating the proteins by immersing the slide in absolute alcohol for about sixteen hours. The section was then stained by the alcohol periodic acid—leukofuchsin method described by Hotchkiss.<sup>2</sup> The staining procedure was performed in a uniform manner in all instances except one in which sections were stained without prior oxidation. Invariably, such sections

3 Hack, M. H. Personal communication to the author.

4 Folch, J., and Uzman, L. L. *Federation Proc.* 7: 155, 1948.

were colorless or nearly so. The treating of the section with reagents prior to alcoholic coagulation and staining was varied in order to study some of the properties of the reactive material of the Golgi apparatus. Enzyme preparations were washed from the section with 5 or 10 drops of the buffer used to dissolve the enzyme, and the section was then coagulated with alcohol and stained as usual. With respect to the methods of treatment, the concentration, the time of action and the effect on the Golgi apparatus are given in the table.

*Effects of Various Extractives and Enzyme Preparations on Golgi Apparatus of Columnar Duodenal Cells of Guinea Pig After Mucosa Had Been Prepared by Freezing and Drying, Sectioned and Treated by Periodic Acid—Leukofuchsin Method*

| Reagent  | Concentration  | Time of Action | Stainability of Golgi Apparatus* |
|--|--|----------------|----------------------------------|
| Acetate buffer $pH$ 3.650                              | M/5  | 1 hr           | +                                |
| Phosphate buffer $pH$ 5.5-8.0                          | M/15   | 1 hr           | +                                |
| Borate buffer $pH$ 8.5-11.0                            | M/5  | 1 hr           | + weak                           |
| Acetic acid ( $pH$ 3.3)                                | 0.2%   | 1 hr           | Very weak                        |
| Acetic acid  | 2.0% 10% glacial   | 1 hr           | —                                |
| Hydrochloric acid                                      | N/10   | 1 hr           | +                                |
| Acetic acid (10%) followed by hydrochloric acid (N/10) |  | 1 hr           | +                                |
| Hydrochloric acid in 30% alcohol                       | N/10   | 1 hr           | +                                |
| Trichloroacetic acid                                   | 5%   | 1 hr           | +                                |
| Propionic acid   |  | 1 hr           | +                                |
| Ammonium sulfide                                       | Saturated  | 15 min         | Weak                             |
| Ammonium hydroxide                                     | 0.0001N  | 1 hr           | Very weak                        |
| Sodium hydroxide                                       | 0.0001N  | 1 hr           | Very weak                        |
| Carbon disulfide                                       |  | 1 hr           | +                                |
| Pyridine   |  | 1 hr           | +                                |
| Dioxane  |  | 1 hr           | +                                |
| Amyl acetate   |  | 1 hr           | +                                |
| Isoamyl alcohol  |  | 1 hr           | +                                |
| Hot methanol chloroform 1:1                            |  | 16 hr          | +                                |
| Saliva in buffer $pH$ 7.0                              | 1:4  | 1 hr           | +                                |
| Pepsin   | 0.1 mg per cc 0.1N HCl                                       | 5 min          | —                                |
|  | 0.2 mg per cc in phosphate buffer $pH$ 8.0                   | 5 min          | —                                |
| Pangestin  | 0.1 mg + 2 mg entero kinase per cc phosphate buffer $pH$ 8.0 | 5 min          | —                                |
| Trypsin  | 2 mg per cc acetate buffer $pH$ 4.0 and 6.0                  | 1 hr           | +                                |
| Emulsin  | 1 mg per cc phosphate buffer $pH$ 7.0                        | 1 hr           | +                                |
| Hyaluronidase  |  | 1 hr           | +                                |
| Small stomach fluid                                    |  | 1 hr           | Very weak                        |
| Cl. welchii toxin                                      | 1 cc per 10 cc phosphate buffer $pH$ 7.0                     | 8 hr           | Very weak                        |

\* A plus sign indicates that the Golgi apparatus was stainable; a negative sign that it was not stainable.

### OBSERVATIONS

Sections of duodenum which were freed of paraffin and coagulated by alcohol show the maximum total amount of reactive groups, which appear more or less red according to their number in specific sites (figs 1 to 5). The epithelium of the glands of Lieberkuhn and of the villi is attached to a thin, rather uniform basement membrane. As in other sites studied (kidney, skin, thyroid gland, lung) this appears as a rather uniform, deeply stained homogeneous

membrane which is continuous with the fainter-staining ground substance of the lamina propria mucosae (figs 2 to 5) The ground substance of the basement membrane infiltrates the network of reticular fibers which lie in it and which are related to those of the lamina propria The columnar epithelial cell is separated from adjacent cells by thin, sharp membranes of cement substance, which appear as pink lines The striated border is deeply stained The colorless nucleus

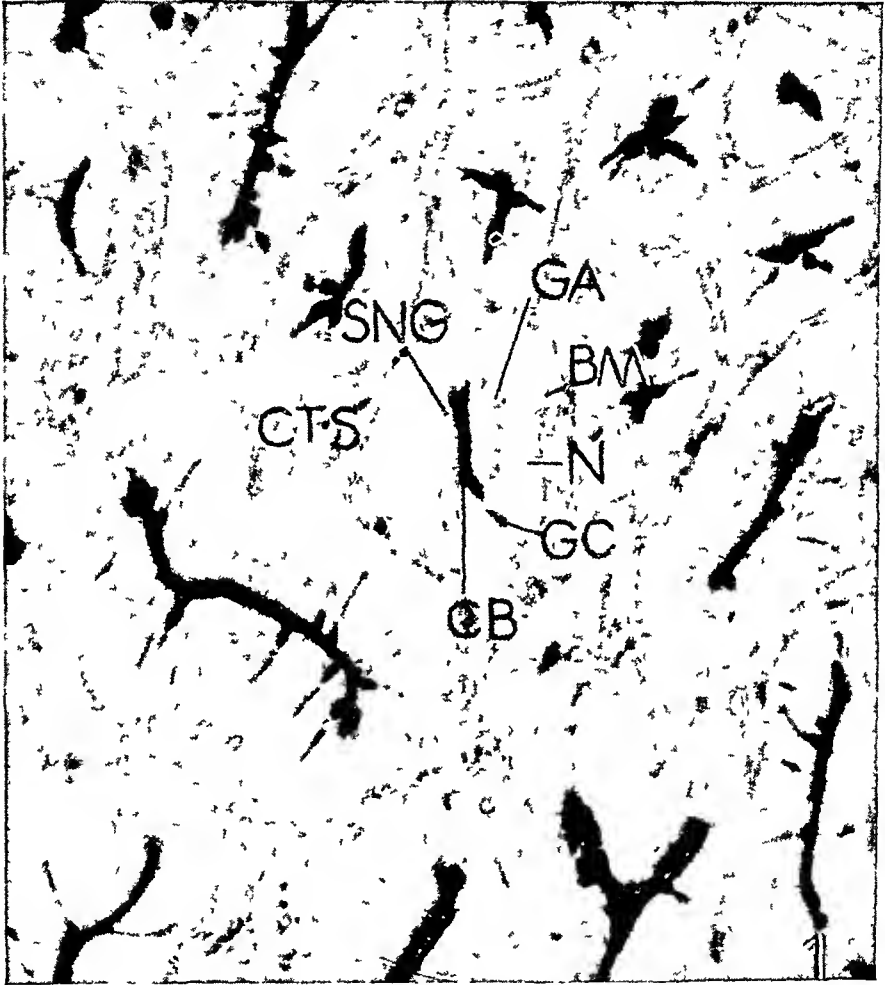


Fig 1—Low power photomicrograph of a transverse section of duodenal glands of the rabbit The specimen was fixed by freezing and drying, sectioned, and stained by the periodic acid—leukofuchsin method GA indicates the Golgi apparatus, SNG, supranuclear granules, CB, the cuticular border, N, a nucleus, CTS, connective tissue stroma, GC, a goblet cell  $\times 250$

lies in cytoplasm which is barely tinted pink with no tendency toward localization Only two cytoplasmic structures stand out sharply, even at low magnifications These are (1) intensely stained small granules lying beneath the striated border and (2) the red to pink Golgi apparatus located between these and the nucleus The Golgi apparatus appears as an irregular fine reticulum or network of red threads 0.3 micron or less in diameter, with thickenings along their extent which are about 0.6 micron or less in diameter No clear "canals" are visible

in this region of the cell, nor are "chromophobic" masses detectable. In sections of the same material which have been stained by common procedures, the portion of the cell occupied by this structure appears as poorly defined clear areas, which constitute the "negative image." The Golgi apparatus stains more deeply in the columnar cells of the glands of Lieberkuhn (figs 2 and 3) than in those of the villus. In addition, the network is more compact and less reticulated in the former than in the latter (figs 4 and 5). It is important to point out that mucigen granules of goblet cells are discrete spherical brilliant-red bodies, exceeding in color density any structure present in the columnar cells.

The behavior of the reactive components of the Golgi apparatus with reagents may be summarized as follows

1 The staining of the Golgi apparatus is not markedly affected by buffers ( $p_H$  3.6 to 8.0), dilute acetic acid (0.2 per cent), dilute hydrochloric acid (tenth normal) or many protein extractives

2 After it has been treated with more concentrated solutions of acetic acid or with glacial acetic acid, the Golgi apparatus cannot be stained. It may be visualized if subsequently treated with hydrochloric acid and stained in the usual manner

3 The Golgi apparatus is weakly stained after extraction has been carried on with dilute sodium or ammonium hydroxide or with ammonium sulfide

4 The Golgi apparatus cannot be stained after the action of pepsin, pangenin or trypsin

5 Although neither emulsin nor hyaluronidase has a notable effect on the intensity of staining of the Golgi apparatus, cytase and *Clostridium welchii* toxin cause marked decrease of its stainability

#### COMMENT

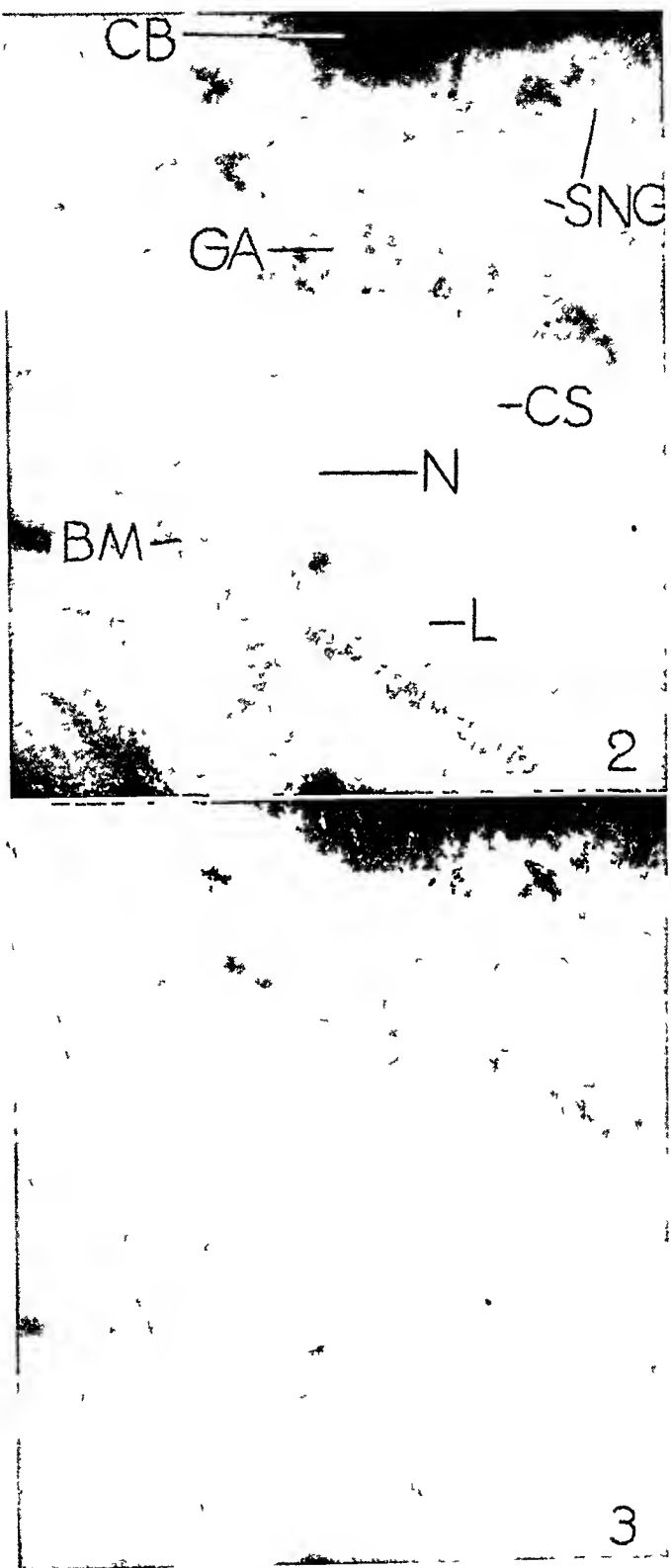
The Golgi apparatus of columnar duodenal cells was described by Cajal,<sup>5</sup> Kopsch<sup>6</sup> and others after it had been visualized by the use of heavy metals. Morphologically, the apparatus which they described corresponds closely to the structure described in the foregoing pages. The chief difference is that with the use of heavy metals the constituent parts are somewhat coarser, possibly because of the effect of the deposition and apposition of reduced metal. Simpson's<sup>7</sup> describing of the Golgi apparatus in columnar cells fixed by freezing and drying as the vaguely defined "negative image" has been confirmed in the material used in this research. There seems little doubt that the structure I have described on the basis of the periodic acid—alcufuchsin method corresponds closely to, or is identical with, the structure which is commonly accepted as the Golgi apparatus

5 Cajal, S. R. *Trab. d. Lab. de invest. biol.* 3: 34, 1904

6 Kopsch, F. *Ztschr. f. mikr.-anat. Forsch.* 5: 221, 1926

7 Simpson, W. L. *Anat. Rec.* 80: 329, 1941





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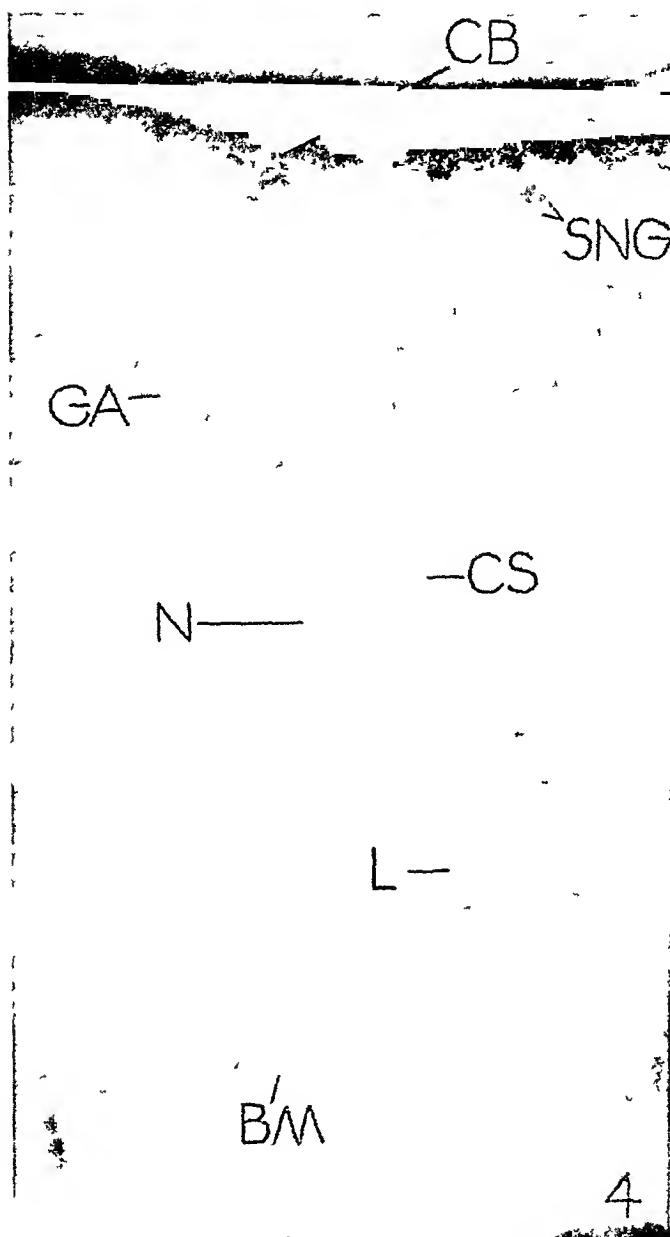


Fig 4—High power photomicrograph of surface epithelium of a villus prepared as described in the legends under the previous figures. In these cells the Golgi apparatus stains paler, and the structural details are finer, than in cells of the glands of Lieberkuhn. The symbols are the same as those for figures 1 and 2  $\times 2,900$

Figs 2 and 3—High power photomicrographs of a portion of a transverse section of a duodenal gland of Lieberkuhn of a rabbit after the tissue had been prepared by freezing and drying, sectioning and staining by the periodic acid—leukofuchsin method. The photomicrographs show the same cells taken at slightly different foci to show the delicacy of detail of the Golgi apparatus. *GA* indicates the Golgi apparatus, *SNG*, supranuclear granules, *CB*, the cuticular border, *N*, nucleus, *CS*, cement substance, *BM*, the basement membrane, *L*, a lymphocyte  $\times 2,900$

The reactive material present in the Golgi apparatus and responsible for visualization with the Hotchkiss method may be characterized, at least tentatively. Since there is no detectable decrement in the intensity

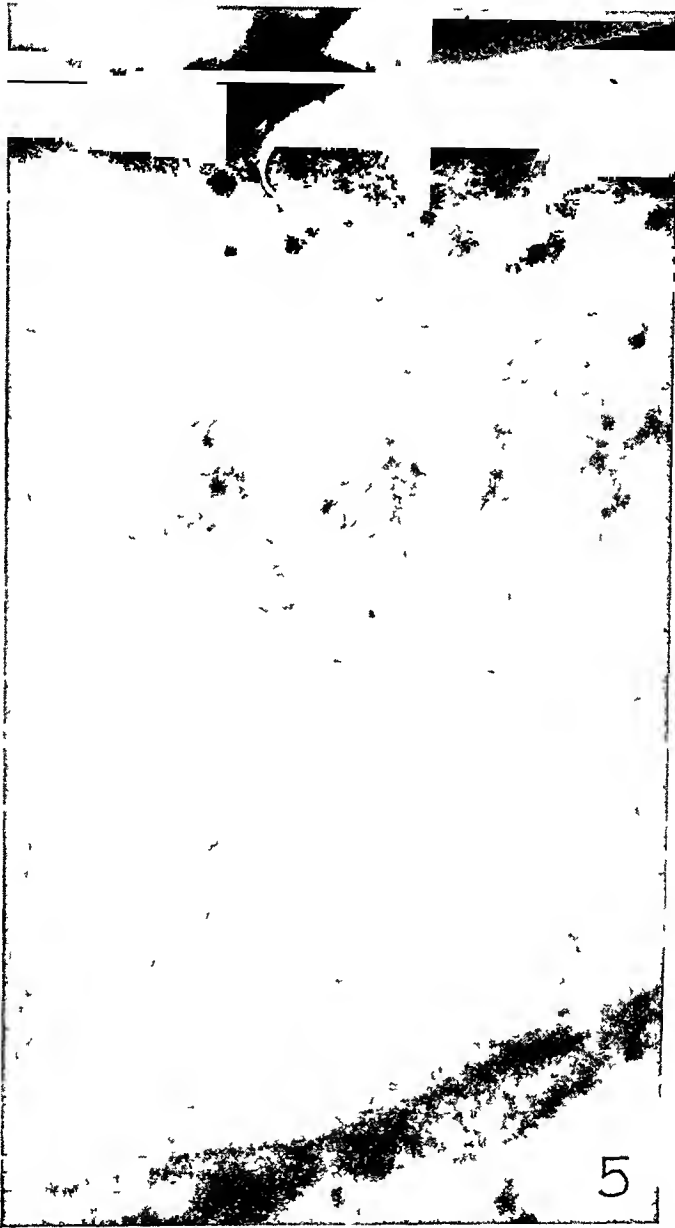


Fig 5—High power photomicrograph of surface epithelium of another villus taken at the same level as that in figure 4  $\times 2,900$

of the staining of the Golgi apparatus after the action of saliva and after extraction with a hot mixture of equal parts of methanol and chloroform, the reactive material may be said to be free of appreciable amounts of glycogen or lipid. This excludes the following classes of lipids (which

give positive results when spot-tested by the periodic acid—leukofuchsin method) as responsible for the colored reaction of the Golgi apparatus kersin, phrenosin, phosphatidyl ethanolamine, acetal phosphatide, inositol phosphatide, lecithin and ganglioside Staining of the Golgi apparatus due to the presence of free aldehydes is excluded by the fact that it does not appear when the section is treated with the leukofuchsin reagent in the absence of prior oxidation by periodate The reactive substance must then be a carbohydrate-containing protein or a lipoprotein The distinction between these classes of proteins is not certain, but a number of lines of evidence suggest that the reactive substance of the Golgi apparatus may be a glycoprotein These lines of evidence are 1 Esterified glycols fail to give a red color after oxidation with periodic acid and treatment with leukofuchsin While it is unusual for acetic acid to form such compounds with simple carbohydrates, the negative reaction I have reported for the Golgi apparatus treated with strong acetic acid or acetic anhydride is understandable only on such a basis The visualization of the Golgi apparatus following the hydrochloric acid treatment of such a negative section would appear, then, to be due to a reconversion of the altered groups to glycols, which are reactive 2 The fact that snail stomach fluid and Cl welchii toxin remove part of the reactive material of the Golgi apparatus clearly indicates the possibility that the reactive material may be a glycoprotein The characteristic action of both of these preparations consists in the depolymerization of polysaccharides In snail stomach fluid, the following enzymes have been identified diastase, invertase, maltase, cellobiase, lipase and the mixture of enzymes known as cytase Proteolytic enzymes are absent<sup>8</sup> The following enzymes have been detected in the toxin of Cl welchii hyaluronidase, lecithinase, collagenase<sup>9</sup> The marked reduction in the stainability of the Golgi apparatus following the action of these mixtures of enzymes indicates that the Golgi apparatus contains a complex polysaccharide Together with the observations that the reactive groups are rapidly removed from the Golgi apparatus by pangestin, pancreatin and pepsin, the foregoing findings suggest that a carbohydrate-protein complex is the principal component of the structure visualized by the periodic acid—leukofuchsin technic 3 It is a striking feature that alkaline buffers, dilute sodium or ammonium hydroxide, and ammonium sulfide reduce the stainability of the Golgi apparatus These reagents also reduce the stainability of the mucigen granules of goblet cells As these granules are considered to consist of glycoproteins, the findings suggest that the reactive groups of the Golgi apparatus contain similar components 4

8 Faberge, A C *Stain Technol* 20 1, 1945 Karrer, P , Staub, M , Weinhagen, A , and Joos, B *Helvet chim acta* 7 144, 1924

9 Oakley, C L , Warrack, G H , and van Heyningen, W E *J Path & Bact* 58 229, 1946

Lipoproteins may possibly be present in the nucleus,<sup>3</sup> which is completely colorless after employment of the test procedure. This indicates that the lipid fraction (or the lipoprotein) of the Golgi apparatus, if present, is either nonreactive or is extracted by the procedure. As knowledge of the chemistry of the poorly characterized class of lipoproteins is still meager,<sup>10</sup> the possibility that some or all of the reactive groups of the Golgi apparatus may be parts of such a complex cannot be excluded at the present time. But, for the reasons cited, it would seem more profitable, at least tentatively, to regard the reactive components of the Golgi apparatus as alcohol groups of a polysaccharide-protein complex.

Certain considerations follow if the Golgi apparatus is regarded as a structure containing polysaccharide. In both plants and animals, polysaccharides are frequently highly polymerized and possess properties of high viscosity or hardness, as, for example, cellulose, chitin, joint fluids, vitreous humor and the ground substance of the connective tissues. All of these structures may be considered ultimately as arising in part from the tendency of the constituent carbohydrate moieties to form submicroscopic structure through polymerization. It is possible that a similar tendency toward the formation of submicroscopic structure may be present in the Golgi apparatus. If this is so, it may be possible to conceive of the Golgi apparatus as a framework whose structure is of such a nature that it may accommodate certain enzymes or other activities in an orderly manner. Thus ascorbic acid<sup>11</sup> and phosphatase(s)<sup>12</sup> which have been described as lying in, or in the region of, the Golgi apparatus may be accommodated within this structure. Also lipid droplets, such as those described by Baker<sup>1</sup> in cells similar to columnar duodenal cells, may exist in relation to this structure, because lipogenetic enzymes may be attached to the glycoprotein structure. Other enzymes, which may be assumed to be involved in protein synthesis, may be attached in an orderly framework and give rise to the large number of prescretion droplets which are said to arise in the meshes of the Golgi apparatus. These speculations may be summarized in the following way. The Golgi apparatus may be a carbohydrate-protein complex, itself relatively inert, which possesses the peculiar submicroscopic structure that provides a suitable framework for the orderly arrangement of enzymes and other activities.

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10 Chargaff, E. Lipoproteins, in Edsall, J. T., and Anson, M. L. *Advances in Protein Chemistry*, New York, Academic Press, 1944, vol. 1.

11 Bourne, G. *Cytology and Cell Physiology*, Oxford, Clarendon Press, 1942.

12 Emmel, V. M. *Anat Rec* **95** 159, 1946. Dempsey, E. W., and Deane, H. W. *J. Cell & Comp. Physiol* **27** 159, 1946. Deane, H. W., and Dempsey, E. W. *Anat Rec* **93** 401, 1945.

Structures having the appearance of the Golgi apparatus have been observed, after employment of the periodic acid—leukofuchsin method, in fibroblasts in various sites and in glandular cells of the thyroid gland. Stainable bodies have also been observed in numerous other types of cells which, though localized in regions containing the Golgi apparatus, do not resemble it in structure. In still other cells no trace of stain has been observed in the region of the Golgi apparatus. This may mean that in different cells the Golgi apparatus contains little or no reactive components (possibly part of a glycoprotein) or that the hydroxyl groups of the polysaccharide exist not in a free or reactive state but rather in a substituted or nonreactive state. Such glycoproteins, if present, might be expected to possess submicroscopic structure and to play the same organizing role which has been postulated for the Golgi apparatus of the columnar cells of the duodenum.

#### SUMMARY

The periodic acid—leukofuchsin method was used to visualize the Golgi apparatus in columnar cells of the duodenum of the rabbit and the guinea pig after this tissue had been prepared by freezing and drying. The stainable component is classified, at least tentatively, as a carbohydrate-protein complex on the basis of the behavior of the reactive material with various reagents and enzyme preparations. The possibility that such a glycoprotein plays a role in cell metabolism is discussed.

# ULCERS OF THE UPPER PART OF THE GASTROINTESTINAL TRACT ASSOCIATED WITH ACUTE DAMAGE OF THE BRAIN

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THE GENESIS of acute esophageal and gastroduodenal ulcers has long been a subject of clinical and experimental interest. As early as 1772, John Hunter<sup>1</sup> believed that the "dissolution of the stomach after death was due to post-mortem continuance of digestion." Hunter postulated a vital principle in all living tissue which prevented autodigestion. Claude Bernard<sup>2</sup> and his pupil Pavy cast doubt on this statement and showed that living tissue could be dissolved in gastric secretion. Recently Price and Lee,<sup>3</sup> by experimenting on gastric digestion in dogs, demonstrated that all living tissue underwent dissolution, in the hyperacid stomach all tissue, including the gastric mucosa, was dissolved more rapidly and more extensively.

Softening of esophagus and stomach in "acute affections of the brain or its membrane" was first described by Rokitsansky<sup>4</sup> in 1849. He postulated that the lesion was probably due "to reflex action of the esophageal and gastric branches of the vagus, or to a diseased innervation of the stomach, owing to morbid condition of the vagus and to extreme acidification of the gastric juice." As Rokitsansky's views have become known, clinicians, as well as experimental investigators, have forged a long chain of evidence connecting hemorrhages and ulcers of the stomach with cerebral lesions. Cushing<sup>5</sup> postulated the neurogenic origin of gastric ulcers and showed that lesions in the region of the third ventricle or the hypothalamus occasionally resulted in gastrointestinal ulceration. Cushing reported 11 cases in which ulcers and erosions of the esophagus and the stomach were associated with intracranial lesions.

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From the Department of Pathology, Toronto East General Hospital

1 Hunter, J. *Philos Tr* 62 47, 1772

2 Bernard, C. *Leçons de physiologie expérimentale*, Paris, 1855-1856

3 Price, P. B., and Lee, T. F. *Surg, Gynec & Obst* 83 61, 1946, 84 959, 1947

4 Rokitsansky, C. *A Manual of Pathologic Anatomy*, London, New Sydenham Society, 1849, vol 2, p 35

5 Cushing, H. *Surg, Gynec & Obst* 55 1, 1932

Mastin and Bunts<sup>6</sup> presented 8 cases of neurogenic erosions and perforations of the stomach and the esophagus in support of Cushing's hypothesis. Boles and Riggs<sup>7</sup> presented 15 cases and brought forward the theory that the acute gastric erosions were due to circulatory insufficiency and had a common basis with the accepted peptic ulcer. They claimed that hypothalamic damage is not always a prerequisite but that increased intracranial pressure may be the necessary stimulus for the production of ulcer.



Fig 1—*A* (case 1), esophagus and stomach showing a perforation of the esophagus and four shallow ulcers and hemorrhagic erosions of the stomach. *B* (case 2), esophagus and stomach showing a perforation of the esophagus and ulcers and hemorrhagic erosions of the stomach. The two ulcers are at the pyloric end and the erosions are along the lesser curvature of the stomach.

In the 4 cases to be reported now, gastric ulceration occurred, and in 3 cases perforation of the stomach and the esophagus followed acute damage of the brain of diverse causation.

#### REPORT OF CASES

CASE 1—J. W., a man aged 63, was brought to the Toronto East General Hospital, Jan 19, 1948, in an unconscious state, having been struck by an auto-

6 Mastin, M. G., and Bunts, R. P. *Arch. Int. Med.* 54:916, 1934.

7 Boles, R. S., and Riggs, H. E. *J. A. M. A.* 115:1771, 1940.



mobile shortly before. A swelling at the back of the head was noted. His breathing was heavy and stertorous. The left side of the face and the left arm were weak, the left leg was flaccid. Knee and ankle reflexes were absent. The pupils were small but equal and reacted sluggishly to light. The patient vomited rather abruptly a large amount of dark brownish fluid. A roentgenogram of the skull showed horizontal linear fractures of the right and left parietal bones.

On admission the patient was still unconscious, restless, moaning and tossing in bed and moving only the right side of his body. He vomited four times in the next twelve hours. The vomiting was projectile, and the vomitus was dark red. In the following two days, suction was utilized, and the return through the suction tube was dark red at all times. His temperature, pulse rate and respiratory rate remained around 102 F, 115 and 34, respectively, for the following three days. The spinal fluid was bloody, with a pressure of 235 mm of water. The left side of his body remained weak. The patient never regained consciousness. He became progressively worse, with profuse sweating and difficult breathing. His temperature rose to 105 F, and death took place three and a half days after admission.

Autopsy, four hours later, revealed an extensive subdural hematoma over the right hemisphere, with laceration and edema of the temporal lobes and the lower part of the frontal lobe. Hypothalamic hemorrhages were noted. There were linear fractures of the left temporal bone extending downward and forward to the basisphenoid and the outer table of the right parietal bone. In the pyloric and lesser curvature regions of the stomach there were four shallow ulcers, each about 1.5 cm in diameter, arranged in a crescentic pattern. The wall of the stomach was greatly dilated. There was a scattering of hemorrhagic erosions in the lower portions of the gastric mucosa. The esophagus had lost its normal mucosal markings and showed a grayish black linear rent measuring 4 by 2 cm and communicating with the mediastinum. This linear rent extended into the cardiac end of the stomach. One hundred cubic centimeters of dark brown gastric contents was found in the posterior mediastinum. This fluid gave a reading of 52 units of free hydrochloric acid. There was marked edema of both lungs, with early bronchopneumonia of the right lung. The pleura of the lower lobe of the right lung was adherent to the diaphragm and the mediastinum.

Histologic examination of the esophagus revealed necrosis and edema of all layers at the edge of the perforation with moderate mobilization of polymorphonuclear and mononuclear leukocytes. The mucosa was denuded, with congestion and hemorrhage of the adjacent tissue. In the erosions of the stomach, the centers of the craters extended down to the submucosa, with sloping edges. The surrounding zone showed edema, congestion, hemorrhage and infiltration with lymphocytes and a minimal number of polymorphonuclear leukocytes.

Microscopic examination of the hypothalamus showed marked congestion, numerous ring hemorrhages and pericellular edema.

CASE 2—L. W., a white woman aged 40, was brought to the Toronto East General Hospital, Jan. 21, 1948, two hours after a sudden collapse at home. She had been perfectly well until 7:30 a.m., when she dropped to the floor unconscious.

The patient was deeply unconscious, not responding to any stimuli. Her temperature, pulse rate and respiratory rate were 98.6 F, 70 and 24, respectively. Blood pressure was 90 mm systolic and 70 mm diastolic. Neurologic findings were normal except for two small hemorrhagic spots on the left disk. The spinal fluid was under pressure, with a hemoglobin reading of 5 per cent, a red blood cell count of 400,000 and a leukocyte count of 600 cells per cubic millimeter. The

patient vomited four times the first day and occasionally during the next few days. The hemoglobin content of the blood was 89 per cent, the red blood cell count was 4,500,000 and the white cell count 9,100 per cubic millimeter. The Kahn test of the blood was positive up to 240 units. The urine indicated moderate proteinuria and showed granular casts. The blood nonprotein nitrogen was 31 mg per hundred cubic centimeters.

This patient was incontinent of urine and feces within four hours after admission. Later she roused slightly from her coma, opened her eyes and moved her right arm first, with slow movement of all limbs following, and responded to moderate stimuli. In the following four days she was extremely restless, the mental state alternated between light sleep, from which she was easily aroused, and wakefulness, when she tossed and struggled.

January 23 the patient had a cough and signs of bronchopneumonia in the lower half of each lung posteriorly. Her temperature went up to 102.8 F, swinging between 100 and 104 F, the pulse rate was 135 to 80, the respiratory rate, 60 to 30. On January 26 her temperature rose to 106 F. Bilateral choking of the disks, bilateral up-going of toes and marked rigidity of the neck were noted. On the last day the spinal fluid was serous pink, with a red blood cell count of 12,000 and a white cell count of 20 per cubic millimeter. Smear and culture revealed no micro-organisms. The patient died at 12:30 a.m., five days after admission.

Autopsy, six hours later, revealed an area of fresh circumscribed hemorrhage, 2 cm in diameter, in the island of Reil on the right side. There was a small softening through the cortex, communicating externally with the subarachnoid space, and there was basal pooling of blood. There was bilateral basal bronchopneumonia with hydrothorax—200 cc of blood-tinged fluid in the left thoracic cavity and coffee-colored fluid in the right—and gastric contents were found in the mediastinum. A perforation in the cardiac end of the esophagus about 6 cm by 2 cm was found. The lower third of the esophagus was soft, partially shredded and slate colored. The stomach was dilated and there were two superficial ulcers, each about 1 cm in diameter, at the pylorus and small hemorrhagic erosions along the lesser curvature.

The cortex at the island of Reil showed extensive hemorrhage. Throughout the leptomeninges there were congestion, moderate perivascular lymphocytic infiltration and early endarteritis. The inflammatory response to the ulcers of the fundus of the stomach and to the esophageal perforation was minimal. A few leukocytes occupied perivascular sites at the margins of the gastric ulcers. The lymphocytic and mononuclear response was greater in the region of the esophageal slough.

CASE 3—F. W., a woman aged 74, was admitted unconscious to a city hospital on March 5, 1948, following a car accident. Her pupils did not react to light. The reflexes were all present. There were lacerations of the nose and over the occipital area and a fracture of the upper thirds of the right tibia and fibula. The patient remained semiconscious, her temperature ranged from 99.2 F on admission to 105 F on March 9 and 10. Her condition steadily declined, and she died five days after the accident.

Autopsy, fifteen hours after death, revealed subdural hemorrhage over the left superoinferior temporal region and deep cerebral laceration and softening of the left suboccipital area, communicating with the lateral ventricle. Fracture of the upper thirds of the right tibia and fibula, basal pneumonia, hyperostosis frontalis interna, pedunculated submucosal lipoma of the pylorus, follicular cysts of the ovaries, and two acute duodenal ulcers, one measuring 2 by 1 cm and the other 0.5 cm in diameter. The esophagus, the stomach and the small and large

bowel otherwise were normal in appearance. There was an organized traumatic hemorrhage replacing the right adrenal gland. Tarry stools were noted in the colon.

The brain revealed edema of the left hemisphere. There were, in addition, a cortical hemorrhage 1 cm in depth in the anterior pole of the left frontal lobe and a deep laceration 5.5 by 3 cm opening into the third ventricle. This laceration involved the left inferior temporal gyrus, the fusiform gyrus and part of the hippocampal gyrus. There was no gross destruction in the hypothalamic region.

Microscopic examination of the superficial ulcers showed excavation down to the muscularis, and the margins were steep. On the floor of the craters there was a zone of desquamated epithelium and cellular debris and an underlying early fibrinoid change. In the adjacent tissue around the ulcers there was a heavy infiltrate of polymorphonuclear leukocytes.

CASE 4—A 12 year old boy was admitted, March 27, 1948, to Toronto East General Hospital for tonsillectomy. He was anesthetized with ethyl chloride followed by ether. During the operation, there was apparently considerable difficulty in maintaining an adequate airway, and the patient became cyanosed at times. At the end of the operation, he suddenly ceased breathing and became deeply cyanosed and the heart stopped beating. Cardiac massage was performed immediately through a left oblique subcostal incision, and at the same time intubation was carried out and artificial respiration started. The heart began to beat again four minutes later, and spontaneous respiration returned. Postoperatively the patient was deeply unconscious, with intermittent muscular twitching and clonic convulsions. He died thirty hours after the accident. His temperature remained around 104 to 106 F, the pulse rate varied between 110 and 160 per minute and the respiratory rate between 30 and 44 per minute during this period of thirty hours of second life.

Autopsy, two hours and fifteen minutes later, revealed marked edema of the brain with a pronounced tentorial and cerebellar cone of pressure. Also discovered were malacia and multiple perforations of the stomach, an acute ulcer of the duodenum and in the left subphrenic space 200 cc of gastric contents with free hydrochloric acid estimated as 16 units, giving a total of 94 units. Other findings were petechiae of the pericardium, the lungs and the thymus.

The stomach showed a dilated fundus. The entire wall except part of the antrum was thin. Multiple erosions and perforations were present. There were five perforations of varying size. Three were located along the greater curvature—one on the anterior surface, 2 by 1 cm, and two on the posterior surface, 3.5 by 1.2 cm and 0.4 cm in diameter, respectively. Two were located along the lesser curvature, one measuring 1 by 0.3 cm and the other 0.2 cm in diameter. The mucous membrane appeared white and greenish gray shreds were adherent to the wall. A few hemorrhagic spots were present over a portion of the pylorus.

Histologic examination of the stomach near the perforation showed digestion and necrosis of all layers. The submucosa was markedly edematous. No cellular infiltration was found. Section of the duodenal ulcer showed that the crater extended down to the muscularis. Focal areas of hemorrhagic erosion surrounded by polymorphonuclear leukocytes were found in the adjacent mucosa. There was extensive edema in the submucosa, with heavy polymorphonuclear infiltration down to the muscularis mucosae.

Tissue from the cortex, the hypothalamus and the basal ganglions showed marked congestion and red cell stasis in the capillaries and larger vessels. The endothelium of a few vessels revealed early degenerative changes with disap-

pearance of cellular structure, while other vascular channels showed mural swelling with prominent large pale cells and nuclei. There was marked distention of the perivascular spaces, which gave a sievelike appearance. The nerve cells were swollen, with distortion of nuclei. The cytoplasm showed liqueficient change with partial to complete loss of tigroid substance. Most of the nerve cells of the cortex were completely degenerated, leaving only their outlines. The nuclei of the oligodendrocytes were moderately swollen and pale. Acute swelling of these cells was well advanced. The astrocytes were swollen and vesicular. The microglia cells were free from nuclear transformation.



Fig 2 (case 4) —Photomicrograph of a neuron of the hypothalamus showing degeneration, with pyknosis of the nucleus, peripheral condensation of tigroid substance and liqueficient change of the cytoplasm. Nissl stain,  $\times 1,640$

#### COMMENT

Erosions and ulcerations of the upper part of the gastrointestinal tract were seen at autopsy in 4 cases of acute craniocerebral damage. These were the only cases in which such lesions were found out of 210 cases of acute fatal head injuries in which autopsy was performed by one of us (Dr Wyatt) in a study of head injuries in the south of England during the last war and more recently as a coroner's pathologist. The major portion of the 210 patients did not survive for a sufficient length of time for gastrointestinal ulcers to develop.

All 4 patients with "neurogenic ulcers" were unconscious from the onset and lived between one and a half and five days. Nutrition was sustained with 5 per cent dextrose in saline solution and other supplements.

administered by intravenous drip. No food was taken by mouth, hence no active digestion of food took place before or at the time of death. The possibility of postmortem digestion of a hyperacid stomach or continuance of digestion in an agonal state was minimized. Two patients who were free of nasopharyngeal bleeding gave evidence of hemorrhage of the upper gastrointestinal tract by vomiting dark bloody gastric contents a few hours after admission. One patient had tarry stools. All of the patients were unconscious, and hence could not feel the pain of perforation. In cases 1 and 2 vomiting with its definite traumatic effect on the esophagus could not be clearly assessed although it is well known that vomiting plays a definite role, as in the Mallory-Weiss<sup>8</sup> syndrome.

In the care of patients with acute cerebral damage there are several clinical features of importance—for instance, the development of an acute pathologic condition of the abdomen, the vomiting of blood or the passage of tarry stools. These features should arouse interest and thought as to the presence of a so-called neurogenic ulcer.

Microscopically, destruction of tissue associated with congestion and thrombosis of submucosal vessels and a variable amount of lymphocytic and polymorphonuclear leukocyte infiltration at the edge of scattered ulcers were found in all 4 cases. The finding of this inflammatory infiltrate was evidence for the antemortem existence of these ulcers.

It is interesting that around the gastric ulcers cellular reaction was minimal to moderate in cases 1 and 2 and absent in case 4, while around the duodenal ulcers there was an extensive concentration of polymorphonuclear leukocytes in cases 3 and 4. The question arises why there was less cellular response to acute gastric ulcers than to duodenal ulcers if they developed at the same time. Morphologically, the duodenal ulcers were of a very early nature.

Several hypotheses can be brought forward. The presence of certain physiologic factors, such as the dissolutive action of the acid gastric juice, may have hastened digestion at the sites of hemorrhagic erosion in the gastric mucosa, with perforation following rapidly before any mobilization of leukocytes was possible, or a chemical factor, such as the acid hydrogen ion concentration of the eroded gastric mucosa, may have exerted an influence such that the mononuclears were left dominant over the polymorphonuclears in the surrounding tissues, or tissue leukocytes may have been inhibited.

The sites of the ulcers of the stomach and the esophagus were examined for evidence of heterotopic alkaline mucosa. No displacement of mucosa was found in any of the cases.

One theory previously propounded was that in unconscious patients there is a resultant atony of the cardiac sphincter allowing the acid gas-

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8 Mallory, G. K., and Weiss, S. *Am J M Sc* 178: 506, 1929.

tric juice to be regurgitated into the esophagus and aiding postmortem digestion. The observation that inflammatory cells, an antemortem stigma of inflammation, were present must be regarded as frank evidence against the postmortem malacia theories in these cases.

Fundamental damage of the brain was common to all the cases, although it varied in origin. In 2 it was of traumatic genesis, in 1 it was due to a spontaneous intracranial hemorrhage with basal pooling of blood and was linked with positive serologic tests indicating syphilis, the fourth case was an example of damage due to anesthetic anoxemia.

Since the time of Cushing the casual relationship of hypothalamic injury and gastric erosions seems to have been well established by experimental workers (Keller, Hare and d'Amour<sup>9</sup>, Watt and Fulton<sup>10</sup>, Hoff and Sheehan<sup>11</sup>), but the exact mechanism of the formation of ulcers is still in dispute. One school (Watt and Fulton<sup>10</sup>, Hoff and Sheehan<sup>11</sup>) has suggested that the gastrointestinal changes are the result of local gastric ischemia due to hyperactivity of the sympathetic vasoconstrictive mechanism. Another school (Cushing<sup>5</sup> and Beattie<sup>12</sup>) advanced the thought that the gastric erosions and hemorrhage are due to stimulation of the parasympathetic center through its efferent fibers contained in the vagus nerve, this nerve conducts the impulse to the gastroduodenal musculature resulting in spasmodic contraction producing ischemia and hemorrhagic erosion leading to ulcer formation. The third school (Boles and Riggs<sup>7</sup>, Penner and Bernheim<sup>13</sup>) expressed the belief that the vasomotor mechanism stimulates peripheral circulatory stasis, leading to ulceration of the gastrointestinal tract. This ulceration results either from central vegetative stimulation through intracranial lesions, or is due to a stimulation of the cranial vegetative center resulting from circulatory failure. These authors also brought forward the thought that systemic disease initially would produce general peripheral stasis with prolonged vasoconstriction and resultant development of acute gastrointestinal ulcers.

These hypotheses are not in conflict fundamentally, as ulceration and hemorrhage have been reported in animals after posterior pituitary injection USP (pituitrin<sup>(R)</sup>) and pilocarpine hydrochloride had been injected into the lateral ventricles (Cushing<sup>14</sup>, Light Bishop and Ken-

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9 Keller, A. D., Hare, W. K., and d'Amour, M. C. *Proc. Soc. Exper. Biol. & Med.* **30** 772, 1933.

10 Watt, J. W., and Fulton, J. J. *Ann. Surg.* **101** 363, 1935.

11 Hoff, E. C., and Sheehan, D. *Am. J. Path.* **11** 789, 1935.

12 Beattie, J. *Canad. M. A. J.* **26** 278, 1932.

13 Penner, A., and Bernheim, A. *Arch. Path.* **28** 129, 1939.

14 Cushing, H. *Proc. Nat. Acad. Sc.* **17** 163 and 239, 1931.

dall<sup>15</sup>), after acetylcholine and betahypophamine (pitressin<sup>(R)</sup>) had been injected intravenously (Necheles and Masur<sup>16</sup>) and after epinephrine hydrochloride had been injected intraperitoneally (Penner and Bernheim<sup>17</sup>) Moreover, Keller,<sup>18</sup> studying the relation of the peripheral nerves and gastric ulcer, observed that bilateral vagotomy performed previous to injury of the hypothalamus resulted in a typical hemorrhage without formation of a crater, while bilateral thoracic and abdominal sympathectomy carried out before injury of the hypothalamus produced erosions without hemorrhage From all these observations it seems that both parasympathetic and sympathetic nerves are concerned in the causation of gastrointestinal ulceration and hemorrhage The present concepts are more in favor of sympathetic overstimulation It is of interest to mention that the bilateral vagotomy<sup>19</sup> advocated for peptic ulcer nowadays has gained some popularity, which further emphasizes the paramount role played by the central nervous system in the production of ulcer

#### SUMMARY

Four cases in which "neurogenic ulcer" occurred with varied types of brain injury are reported In 4 cases criteria of antemortem inflammation were presented The pathogenesis and experimental contributions bearing on it are reviewed From the point of view of care and treatment, awareness of the condition is important in all cases of acute cerebral injury

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15 Light, R A , Bishop, C E , and Kendall, L E J Pharmacol & Exper Therap 45 222, 1932

16 Necheles, H , and Masur, W Am J Digest Dis 6 389, 1939

17 Penner, A , and Bernheim, A I J Exper Med 70 453, 1939

18 Keller, A D ~ Arch Path 21 165, 1936

19 Dragstedt, L R Surg , Gynec & Obst 83 547, 1946 Torek, P J A M A 135 1141, 1947

# DIET AND CIRRHOSIS OF THE LIVER

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**B**Y THE needle biopsy technic (Wahi<sup>1</sup>) it has been possible to study histologically livers of a large number of patients who were admitted to the Thomason Hospital, Agra, for varied symptom complexes. During the study I have been impressed with the frequency of hepatic injury of varying grades in patients not primarily admitted for disease of the liver. Cirrhosis of the liver is common among Indians, and the patients with this disease are usually drawn from two classes of society, orthodox Hindus, who are strict vegetarians, and the poorer classes, who eat what they grow or what is cheapest. The diets of both these sections of the population are ill balanced.

Nutritional disease of the liver can be produced in animals by a variety of means and the resulting lesions have attracted great attention in recent years. Although some workers have been overenthusiastic in applying what they have observed in animals to the diseases of human beings, any relationship between the experimentally produced disease of the liver and that occurring in man is far from established.

## REVIEW OF THE LITERATURE

That diet influences the degree to which the liver is sensitive to injury is no new discovery. Opie and Alford,<sup>2</sup> in 1914, and Davis and Whipple,<sup>3</sup> in 1919, showed that the degree to which their animals were susceptible to chloroform poisoning depended largely on the character of the diet they were receiving. In the years following, the work was directed largely toward determining the influence of diet on the hepatic injury caused by exposure to definite hepatic poisons. Weichselbaum<sup>4</sup> in 1935 was the first to demonstrate that dietary factors alone can initiate injury of the liver. He noticed that a dietary deficiency of protein, par-

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1 Wahi, P. N. *Indian M. Gaz.* **31** 130, 1946.

2 Opie, E. L., and Alford, L. B. *J. A. M. A.* **62** 895, 1914.

3 Davis, N. C., and Whipple, G. H. *Arch. Int. Med.* **23** 612, 1919.

4 Weichselbaum, T. M. *Quart. J. Exper. Physiol.* **25** 363, 1935.



ticularly of sulfur-containing amino acids, caused death with hemorrhage of the liver. Since then, hepatic injury resulting from dietary deficiencies has attracted increasing attention, and new information concerning the causation of hepatic disease has been acquired. It may therefore be opportune to review and reorient present concepts of hepatic damage in the light of the experimental findings.

#### EXPERIMENTAL PRODUCTION OF LESIONS IN THE LIVERS OF ANIMALS BY DEFICIENT DIETS

*Diets Deficient in Proteins*—Hepatic lesions produced by dietary deficiency did not attain prominence until 1940, when Rich and Hamilton<sup>5</sup> reported that cirrhosis of the liver had been produced in rabbits by a synthetic diet lacking in yeast but supplemented by vitamins. The same authors<sup>6</sup> described cirrhosis of the liver resembling Laennec's cirrhosis of man occurring in all of 14 rabbits kept from 25 to 113 days on diets lacking yeast but supplemented by various vitamins. In some of the animals ascites also developed. They concluded that cirrhosis was due to the lack of some factors of yeast other than thiamine, riboflavin, pyridoxine and nicotinic acid. Probably it was choline. These findings became all the more interesting in view of the observations of Von Glahn and Flinn<sup>7</sup> that yeast added to the diet of rabbits reduces somewhat the tendency of liver to be damaged by lead arsenate.

Lillie, Daft and Sebrell<sup>8</sup> produced cirrhosis of the liver in rats with a diet which was low in protein (4 to 10 per cent) and in sulfur-containing amino acids but which was supplemented with thiamine, nicotinic acid, pyridoxine and pantothenic acid. The fat content of this diet varied from 5 to 70 per cent. They found that on the average the pathologic changes were more severe when approximately 20 per cent alcohol was substituted for drinking water. Lowry and co-workers<sup>9</sup> reported that treating with choline or increasing the casein content of the diet to 50 per cent resulted in hyperplastic regeneration of the liver and clinical improvement of rats with experimentally produced cirrhosis of the liver. In 1942 Lillie and associates,<sup>10</sup> describing the histologic aspects and the

5 Rich, A. R., and Hamilton, J. D. *Tr. A. Am. Physicians* **55** 133, 1940.

6 Rich, A. R., and Hamilton, J. D. *Bull. Johns Hopkins Hosp.* **66** 185, 1940.

7 Von Glahn, W. G., and Flinn, F. B. *Am. J. Path.* **15** 771, 1939.

8 Lillie, R. D., Daft, F. S., and Sebrell, W. H. *Pub. Health Rep.* **56** 1255, 1941.

9 Lowry, J. V., Daft, F. S., Sebrell, W. H., Ashburn, L. L., and Lillie, R. D. *Pub. Health Rep.* **56** 2216, 1941.

10 Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. *Pub. Health Rep.* **57** 502, 1942.

pathogenesis of cirrhosis of the liver, stated that among the noteworthy features is the absence of hemorrhage and, in the earlier stages, obvious necrosis of liver cells

Gillman and co-workers<sup>11</sup> and Gillman and Gillman<sup>12</sup> found, from studies of biopsy specimens of liver, that extensive damage of the liver is invariably present in cases of malnutrition, including pellagra and nutritional edema, whether the patients are children or adults. Gilbert and associates,<sup>13</sup> Gillman,<sup>14</sup> Gilbert and Gillman<sup>15</sup> and Gillman and co-workers<sup>16</sup> showed that severe damage of the liver develops in young rats fed ad libitum on a diet of mealie pap (maize meal porridge) and sour milk. It expresses itself as diffuse fatty liver, cirrhosis, lobar absorption or diffuse lobar enlargement. One or more of these conditions may be present in the same liver. Mealie pap, supplemented frequently by fermented whole cow's milk, is the staple diet of many South African Negroes, it has a high carbohydrate and low protein content.

Himsworth and Glynn<sup>17</sup> reported that massive hepatic necrosis was produced in rats by low protein diets. They found that proteins vary in their power to prevent the lesion, indicating thereby that the protective factor is a component of protein rather than the intact protein molecule itself. They<sup>18</sup> further found that massive hepatic necrosis due to a protein-deficient diet is prevented in rats by adding either casein (8 per cent) or an equivalent amount of dl-methionine to the diet. The lesion was not prevented by l-cystine in an amount equivalent to that contained in an 8 per cent casein diet. A daily supplement of 4 mg of choline to each animal or a combination of choline and cystine in that amount was equally ineffective. They came to the conclusion that the protective action of casein is due to the methionine contained in it. They found no evidence that hepatic necrosis results from vitamin or mineral deficiency.

Fouts<sup>19</sup> reported the development of a deficiency state characterized by loss of weight, moderate anemia, dermal and peptic ulcers, a fatty

11 Gillman, T, Gillman, J, Inglis, J, Friedlander, L, and Hammar, E  
Nature, London 154 210, 1944

12 Gillman, J, and Gillman, T Lancet 2 161, 1944

13 Gilbert, C, Gillman, J, Mandelstam, J, Gillman, T, and Golberg, L  
South African J M Sc 8 148, 1943

14 Gillman, J Brit M J 1 149, 1944

15 Gilbert, C, and Gillman, J Science 99 398, 1944

16 Gillman J, Gillman, T, Mandelstam, J, and Gilbert, C Brit J  
Exper Path 26 67, 1945

17 Himsworth, H P, and Glynn, L E (a) Clin Sc 4 421, 1942 (b)  
5 93, 1944, (c) Lancet 1 457, 1944

18 Himsworth, H P, and Glynn, L E Clin Sc 5 133, 1944

19 Fouts, P L J Nutrition 25 217, 1943

cirrhotic liver and death in dogs fed a diet low in protein (15 per cent casein) but supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine and pantothenic acid. An increase in the amount of protein (41 per cent) prevented the condition.

Li and Freeman<sup>20</sup> reported that fatty liver developed in dogs maintained on a diet that was 33 per cent fat and deficient in protein. Waterlow,<sup>21</sup> discussing the etiologic factors of the so-called "fatty liver disease" of West Indian infants, pointed out that all the evidence, including the history, the associated lesions and the response to treatment, suggested that the underlying deficiency was either in protein or in some member of the vitamin B complex.

*Diets Deficient or Ill Balanced in Amino Acids*—Since the recognition of fatty infiltration of the liver as a definite precirrhotic condition, the lipotropic activity of various amino acids has been a subject of intense investigation. The existence of lipotropic factors was first indicated by Hershey<sup>22</sup> and Hershey and Soskin,<sup>23</sup> while they were studying the development of fatty liver in pancreatectomized dogs maintained on insulin. Accepting the view that phospholipids were essential for fat transport, Hershey<sup>22</sup> fed his animals lecithin and noted marked reduction of liver fat. Best, Hershey and Huntsman<sup>24</sup> and Best and Huntsman<sup>25</sup> showed that addition of choline, a constituent of phospholipids, brought the livers of their rats to normal. Both choline and lecithin were found to prevent the accumulation of fat and to effect its mobilization from an already infiltrated organ. These results have been corroborated by Best and Ridout<sup>26</sup> and Best, Channon and Ridout,<sup>27</sup> who demonstrated a similar effect for cholesterol deposited in the liver.

The lipotropic activity of various proteins has since been investigated. Besides lecithin and choline, casein was found to be lipotropic, exerting its maximum effect when it constituted 30 per cent of the diet (Best and Huntsman<sup>28</sup>, Channon and Wilkinson<sup>29</sup>). The list of proteins exerting lipotropic action has now been extended to include egg white and beef

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20 Li, T W, and Freeman, S. *Am J Physiol* 145 646, 1946

21 Waterlow, J C. *Proc Roy Soc Med* 40 347, 1947

22 Hershey, J M. *Am J Physiol* 93 657, 1930

23 Hershey, J M, and Soskin, S. *Am J Physiol* 98 74, 1931

24 Best, C H, Hershey, J M, and Huntsman, M E. *J Physiol* 75 56, 1932

25 Best, C H, and Huntsman, M E. *J Physiol* 75 405, 1932

26 Best, C H, and Ridout, J H. *J Physiol* 78 415, 1933

27 Best, C H., Channon, H J, and Ridout, J H. *J Physiol* 81 409, 1934

28 Best, C H, and Huntsman, M E. *J Physiol* 83 255, 1935

29 Channon, H J, and Wilkinson, H. *Biochem J* 29 350, 1935

muscle powder (Best, Grant and Ridout<sup>30</sup>) and edestin, fibrin and gliadin (Channon and co-workers<sup>31</sup>) On the other hand, lysine, glutamic and aspartic acids, glycine, alanine, valine, serine, phenylalanine, proline, hydroxyproline, leucine, histidine, arginine and cystine have no lipotropic action (Beeston and Channon,<sup>32</sup> Beeston, Channon and Platt,<sup>33</sup> Beeston and Platt<sup>34</sup>) Cystine has been found to be alipotropic, its use leads to further accumulation of fat in the livers of animals fed a fatty diet The studies of Gyorgy, Poling and Goldblatt,<sup>35</sup> Blumberg and McCollum<sup>36</sup> and Earle and Victor<sup>37</sup> showed that extra cystine added to the experimental diets accelerated the development of cirrhosis

Tucker and Eckstein<sup>38</sup> suggested that the lipotropic activity of a protein was due to the opposing effects of the cystine and the methionine contained in it The lipotropic activity of methionine was confirmed by Channon, Platt and Smith,<sup>39</sup> Best and Ridout<sup>40</sup> and Best and Lucas<sup>43</sup> The effect of protein on fat transport has been satisfactorily explained as being due to the supply of methyl groups for the synthesis of choline (McHenry and Patterson<sup>42</sup>) This has been further proved by studies of the metabolism of methionine in which labeled elements, like heavy hydrogen and radioactive sulfur, were used, which showed that the lipotropic action of methionine is associated with its methyl group This is transferred intact in the synthesis, within the body, of such methyl compounds as creatine, sarcosine and choline (DuVigneaud and associates<sup>43</sup>) Griffith<sup>44</sup> showed that in young rats fed a diet low in

30 Best, C H , Grant, R , and Ridout, J H J Physiol 86 337, 1936

31 Channon, H J , Loach, J V , Loizides, P A , Manifold, M C , and Soliman, G Biochem J 32 976, 1938

32 Beeston, A W , and Channon, H J Biochem J 30 280, 1936

33 Beeston, A W , Channon, H J , and Platt, A P J Soc Chem Indust 56 292, 1937

34 Beeston, A W , and Platt, A P J Soc Chem Indust 58 557, 1939

35 Gyorgy, P , Poling, E C , and Goldblatt, H Proc Soc Exper Biol & Med 47 41, 1941

36 Blumberg, H , and McCollum, E V Science 93 598, 1942

37 Earle, D P , and Victor, J J Exper Med 73 161, 1941

38 Tucker, H F , and Eckstein, H C J Biol Chem 121 479, 1937

39 Channon, H J , Platt, A P , and Smith, J A B Biochem J 31 1736, 1937

40 Best, C H , and Ridout, J H J Physiol 97 489, 1940

41 Best, C H , and Lucas, C C , in Harris, R S , and Thimann, K V Vitamins and Hormones Advances in Research and Applications, New York, Academic Press, Inc , 1943, vol 1, p 1

42 McHenry, E W , and Patterson, J M Physiol Rev 24 128, 1944

43 DuVigneaud, V , Chandler, J P , Cohn, M , and Brown, G B J Biol Chem 134 787, 1940 DuVigneaud, V , Cohn, M , Chandler, J P , Schenck, J R , and Simmonds, S ibid 140 625, 1941

44 Griffith, W H J Nutrition 21 291, 1941

choline hemorrhagic degeneration was absent or slight if the diet contained more than 0.9 per cent of methionine, regardless of whether this level was attained by administering supplementary methionine or by insuring protein high in casein, lactalbumin or fibrin. Thus choline is a dietary essential if the ration contains less than 0.8 per cent methionine. Choline requirements vary inversely with dietary methionine and are increased by dietary cystine.

The alipotropic action of cystine was investigated by Beeston and Channon,<sup>32</sup> who drew attention to an observation of Curtis and Newburgh<sup>45</sup> that when cystine constituted 0.75 to 20 per cent of the diet it produced in addition to necrosis a large increase of fat. There is no agreement on the manner in which cystine exerts its alipotropic effect. Best and Lucas<sup>41</sup> thought that this action was probably nonspecific and due to the raising of metabolism nearer to normal, so that there was increased demand for lipotropic factors. On the other hand, Treadwell and co-workers<sup>46</sup> indicated the possibility that there was direct antagonism between methionine and cystine. An excess of fat accumulated in the liver is an evidence of an alteration of metabolism due either to failure of the transport of hepatic fat or to a withdrawing of fat from body stores so rapidly that the liver is unable to cope with the fat brought to it. Mulford and Griffith<sup>47</sup> showed that in a diet containing 18 per cent casein the sulfur level is suboptimal for the growth of rats (0.14 instead of 0.19 per cent) and that a supplement of sulfur (0.05 per cent) as cystine causes increased growth without corresponding increased intake of food. They suggested that in this process methionine is diverted to form tissue proteins and is thus deprived of its lipotropic action. For a detailed discussion of the mode of action of lipotropic and alipotropic factor the reader is referred to an excellent review by Glynn.<sup>48</sup>

*Diets Rich in Fats*—Besides protein deficiency, diets rich in fats have been reported to cause damage of the liver. Chaikoff and associates<sup>49</sup> demonstrated that cirrhosis of the liver occurs in completely depancreatized dogs. A constant finding in the liver was an early increase of the hepatic fat. The sequence of events was fatty infiltration, hyaline degeneration, atrophy of the cells at the periphery of the lobule, and fibroblastic proliferation ending in a typical hobnail liver. In such animals fatty infiltration of the liver is probably due to the absence of

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45 Curtis, A. C., and Newburgh, L. H. *Arch. Int. Med.* **39**: 828, 1927.

46 Treadwell, C. R., Groothuis, M., and Eckstein, H. C. *J. Biol. Chem.* **142**: 653, 1942.

47 Mulford, D. J., and Griffith, W. H. *J. Nutrition* **23**: 91, 1942.

48 Glynn, L. E. *Nutrition Abstr. & Rev.* **16**: 751, 1947.

49 Chaikoff, I. L., Connor, C. L., and Biskind, G. R. *Am. J. Path.* **14**: 100, 1938.

a pancreatic enzyme which enables methionine to act as a lipotropic factor (Chaikoff, Entenman and Montgomery<sup>50</sup>)

Blumberg and Grady<sup>51</sup> observed cirrhosis of the liver of the diffuse nodular type in rats fed large quantities of wheat germ oil or corn oil for approximately 200 to 400 days. A large oil supplement was added to the basal ration so that the protein content was reduced to about 10 per cent and the fat increased to about 50 per cent. This resulted in prolonged fatty infiltration followed by cirrhosis. The addition of choline prevented the production of cirrhosis.

That fatty infiltration of the liver is an important precursor of cirrhosis of the liver has been shown by many workers. Cholesterol has long been known to increase the lipid content of the liver (Best and Ridout<sup>52</sup>). Cirrhosis has been observed in rabbits after the administration of cholesterol by Chalutow<sup>53</sup> and more recently by Leary<sup>54</sup>. Connor<sup>55</sup> showed that long-continued fatty infiltration of the liver is a factor of great importance in the development of cirrhosis in diabetes and chronic alcoholism. In both conditions he attributed it to an alteration of the metabolism of the hepatic carbohydrate and fat. Blumberg and McCollum<sup>56</sup> reported that cirrhosis of the liver developed in more than 300 rats fed a high fat (55 to 70 per cent) and low protein (10 per cent casein) diet. By the addition of choline they prevented its development in a like number of animals.

Webster<sup>56</sup> and Handler and Dubin<sup>57</sup> described necrosis and cirrhosis of the liver in rats receiving diets poor in protein and choline but rich in fat. The hepatic lesions were prevented by increasing the protein content of the diet (casein from 8 to 18 per cent) and by the addition of molasses. A reduction in the fat content or the addition of betaine diminished the severity of the lesion, while the addition of cystine and cholesterol increased it.

Himsworth and Glynn<sup>18</sup> reported that diffuse hepatic fibrosis (portal cirrhosis) was produced by diets containing an excess of fat (51 per cent) and leading to long-continued fatty infiltration of the liver. Since the

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50 Chaikoff, I. L., Entenman, C., and Montgomery, M. L. *J. Biol. Chem.* **160** 489, 1945.

51 Blumberg, H., and Grady, H. G. *Arch. Path.* **34** 1035, 1942.

52 Best, C. H., and Ridout, J. H., in Luck, J. M. *Annual Review of Biochemistry*, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 349.

53 Chalutow, S. S. *Beitr. z. path. Anat. u. z. allg. Path.* **57** 85, 1914.

54 Leary, T. *Arch. Path.* **32** 507, 1941.

55 Connor, C. L. (a) *Am. J. Path.* **14** 347, 1938. (b) *J. A. M. A.* **112** 387, 1939.

56 Webster, G. T. *J. Clin. Investigation* **21** 385, 1942.

57 Handler, P., and Dubin, I. N. *J. Nutrition* **31** 141, 1946.

diffuse hepatic fibrosis depended on the preceding prolonged fatty infiltration, a diet low in lipotropic factors had the same effect as the one rich in fat

*Diets Deficient in Vitamins*—Vitamin deficiencies have also been charged with the production of hepatic injury Gyorgy and Goldblatt<sup>58</sup> observed various pathologic changes in 48 rats kept on a basal diet deficient in the vitamin B complex but supplemented with thiamine and riboflavin or with thiamine, riboflavin and pyridoxine These changes were characterized by parenchymatous and fatty degeneration, focal and massive necrosis, hyperemia and hemorrhage and, in some rats, by perilobar and condensation fibrosis The addition of yeast or a yeast extract (Peter's<sup>58a</sup> eluate) prevented the hepatic injury, while the condition was aggravated by l-cystine They came to the conclusion that the hepatic changes were dependent on a deficiency of a part of the "vitamin B<sub>2</sub> complex" Later the same authors<sup>59</sup> reported that it was deficiency of choline in rats kept on the basal ration commonly used in the study of the "vitamin B<sub>2</sub> complex" which was responsible for the production of fatty infiltration of the liver They could prevent fatty infiltration by adding 2 mg of choline per gram of the experimental diet or by the addition of dl-methionine

It is an accepted view that certain of the "B vitamins" influence the fat content of the liver McHenry<sup>60</sup> reported that thiamine encouraged the development of fatty liver in choline deficiency, probably by converting carbohydrate to fat Halliday<sup>61</sup> attributed lipotropic activity to pyridoxine (B<sub>6</sub>), but this was disputed by Gavin and McHenry<sup>62</sup> Their failure to produce fatty liver with pyridoxine-deficient diets may be due to the shorter periods of their feeding (Engel<sup>63</sup>) Biotin, on the other hand, causes hepatic deposition of fat (McHenry and Gavin<sup>64</sup>), an effect which is inhibited by inositol but not by choline (Gavin and Mc-

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58 Gyorgy, P, and Goldblatt, H J Exper Med 70 185, 1939

58a Kinnersly, H W, O'Brien, J R P, Peters, R A, and Reader, V Biochem J 27 225, 1933

59 Gyorgy, P, and Goldblatt, H (a) J Exper Med 72 1, 1940, (b) Proc Soc Exper Biol & Med 46 492, 1941, (c) J Exper Med 75 355, 1942

60 McHenry, E W J Physiol 86 27, 1936, Science 86 200, 1937

61 Halliday, N J Nutrition 16 285, 1938

62 Gavin, G, and McHenry, E W J Biol Chem 132 41, 1940

63 Engel, R W J Nutrition 24 175, 1942

64 McHenry, E W, and Gavin, G Proc Am Soc Biol Chem 35 lxxxvii, 1941

Henry<sup>65</sup>) Best and co-workers<sup>66</sup> confirmed the lipotropic action of inositol but failed to find any evidence of an action inhibitory of biotin

It is probable that both fatty infiltration and vitamin deficiency play a role in the development of cirrhosis of the liver (Greene<sup>67</sup>) Spellberg and Keeton<sup>68</sup> produced fatty degeneration of the liver in guinea pigs and rabbits with diets lacking in vitamin C but otherwise adequate However, this factor was not present in sufficient amounts in the orange juice given for its antiscorbutic effects, as neither ascorbic acid nor orange juice had any prophylactic effect on the pathologic changes They suggested the possibility of some unknown factor, absent from the artificial diet, which may be present in the animal's normal diet containing leafy vegetables and carrots

Pancreas and yeast both contain lipotropic substances Best and Ridout<sup>69</sup> attributed the lipotropic effect of pancreas to its content of choline, methionine and inositol Another unidentified factor, "lipocaine," has also been described (Dragstedt, Van Prohaska and Harms<sup>70</sup>, Van Prohaska, Dragstedt and Harms<sup>71</sup>) Recently, Chaikoff, Entenman and Montgomery<sup>50</sup> suggested that the lipotropic activity of pancreatic extracts is due to the methionine liberated from dietary protein That yeast can prevent cirrhosis was shown by Webster,<sup>72</sup> who prevented hepatic cirrhosis in rats by administering large amounts of yeast or molasses However, the nature of its action is unknown, although Himsworth and Glynn<sup>17b</sup> expressed the belief that this is due to its lipotropic property

*Influence of Diet in Modifying Hepatic Injury Due to Hepatotoxic Agents*—Opie and Alford<sup>2</sup> were the first to demonstrate that the degree to which animals were susceptible to chloroform poisoning depended largely on their diet A high carbohydrate diet afforded considerable protection, while meat was less effective The protective value of carbohydrates was confirmed in 1919 by Davis and Whipple<sup>3</sup> when they demonstrated definite diminution of resistance to chloroform poisoning in starving animals

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65 Gavin, G, and McHenry, E W J Biol Chem 141 619, 1941

66 Best, C H, Lucas, C C, Patterson, J M, and Ridout, J H Biochem J 40 368, 1946

67 Greene, C H J A M A 121 715, 1943

68 Spellberg, M A, and Keeton, R W Am J M Sc 200 688, 1940

69 Best, C H, and Ridout, J H Am J Physiol 122 67, 1938

70 Dragstedt, L R, Van Prohaska, J, and Harms, H P Am J Physiol 117 175, 1936

71 Van Prohaska, J, Dragstedt, L R, and Harms, H P Am J Physiol 117 166, 1936

72 Webster, G Tr A Am Physicians 55 139, 1940



A high fat diet has an adverse effect on the resistance of the liver Schiffrin<sup>73</sup> reported that hepatic necrosis produced in dogs with arsphenamine was maximum in animals on a high fat diet Messinger and Hawkins<sup>74</sup> obtained the same results with arsphenamine Himsworth and Glynn,<sup>17a</sup> while investigating the effect of diet on the livers of rats poisoned with trinitrotoluene, observed that the animals fed a high fat diet fared badly, while those fed diets high in protein or high in carbohydrate enjoyed almost complete protection Bollman<sup>75</sup> found that when extensive hepatic necrosis was produced in rats by repeated exposure to carbon tetrachloride, the greatest amount of damage was present in fat-fed rats and the least in rats fed carbohydrates Regenerative changes were most evident in the animals receiving protein diets

Lipotropic agents have not been found to exert any appreciable protective action against hepatotoxic substances such as chloroform (Miller, Ross and Whipple<sup>76</sup>), phosphorus (Best, MacLean and Ridout<sup>77</sup>) and carbon tetrachloride (Barrett and associates<sup>78</sup>, Post and associates<sup>79</sup>) However, Von Glahn and Flinn<sup>7</sup> reported that yeast added to the diet of rabbits reduces somewhat the tendency of the liver to be damaged by lead arsenate, and Suguira and Rhoads<sup>80</sup> reported protection against para-dimethylaminoazobenzene

Sometimes a diet with a high fat content seems to exert protective action against necrosis due to deficiency of sulfur-containing amino acids or that due to exogenous poisons (Gyorgy and Goldblatt<sup>59c</sup>, Himsworth and Glynn<sup>17b</sup>) Turnbull<sup>81</sup> pointed out the tendency of liver cells containing fat to be spared in poisoning with trinitrotoluene Smith<sup>82</sup> observed that rats fed a high fat diet showed less hepatic injury in experimental poisoning A similar observation was made by Earle and Victor<sup>83</sup> in hepatic injury produced by a high intake of cystine

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73 Schiffrin, A Virchows Arch f path Anat **287** 175, 1932

74 Messinger, W J, and Hawkins, W B Am J M Sc **199** 216, 1940

75 Bollman, J L J A M A **121** 1413, 1943

76 Miller, L L , Ross, J F, and Whipple, G H Am J M Sc **200** 739, 1940

77 Best, C H , MacLean, D L, and Ridout, J H J Physiol **83** 275, 1935

78 Barrett, H M , Best, C H , MacLean, D L, and Ridout, J H J Physiol **97** 103, 1939

79 Post, J , Earle, D P , Patek, A J, Jr, and Victor, J Am J Path **18** 661, 1942

80 Suguira, K, and Rhoads, C P Cancer Research **1** 3, 1941

81 Turnbull, H M Proc Roy Soc Med **10** 47, 1917

82 Smith, M I Pub Health Rep **54** 1441, 1939

83 Earle, D P, and Victor, J J Exper Med **75** 179, 1942

That protein also plays a part in modifying the damage of the liver has been pointed out recently by Goldschmidt, Vars and Ravdin<sup>84</sup> in rats in which protein diminished the extent of chloroform necrosis. Miller and Whipple<sup>85</sup> noted that the injury produced in the livers of dogs by chloroform anesthesia was directly proportional to protein depletion. Similar observations have been reported in regard to arsphenamine and trinitrotoluene poisoning (Schiffrin<sup>73</sup>, Messinger and Hawkins<sup>74</sup>, Himsworth and Glynn<sup>17a</sup>). Smith, Lillie and Stohlman<sup>86</sup> observed that high protein diets protected against para-aminoazobenzene and dimethylaminoazobenzene. Maxon<sup>87</sup> and Smith<sup>82</sup> demonstrated a similar protective action against selenium.

*Types of Hepatic Lesions Produced in Animals by Dietary Deficiency*

—It is only within the past few years that lesions of the liver resulting from dietary deficiency have attracted attention. The hepatic lesions produced by diet alone or by diet plus poisons, toxins or infections can be grouped as follows:

**Necrosis** A massive acute necrosis, either diffuse (simulating acute yellow atrophy of man) or focal, with or without fatty changes, and with or without hemorrhage, has been produced by various workers and is the most striking lesion produced by dietary deficiency (Gyorgy and Goldblatt<sup>88</sup>, Cameron and Karunartne<sup>89</sup>, Earle and Victor<sup>37</sup>, Himsworth and Glynn<sup>17b</sup>, Glynn and Himsworth<sup>90</sup>). Lesions observed by Weichselbaum<sup>4</sup> in rats fed a low cystine diet were probably of the same nature, although he described them only as hemorrhages. Recent work of Himsworth and Glynn<sup>18</sup> and Glynn, Himsworth and Neuberger<sup>91</sup> indicates that necrosis is not necessarily the sequel to fatty infiltration but is the result of thioaminoacid deficiency.

**Fatty Infiltration** This is usually produced by diets low in protein and high in neutral fat (Handler and Bernheim<sup>92</sup>, Glynn<sup>48</sup>) and is probably due to deficiency of choline or of amino acids like methionine.

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84 Goldschmidt, S., Vars, H. M., and Ravdin, I. S. *J. Clin. Investigation* **18** 277, 1939.

85 Miller, L. L., and Whipple, G. H. *Am. J. M. Sc.* **199** 204, 1940.

86 Smith, M. I., Lillie, R. D., and Stohlman, E. F. *Pub. Health Rep.* **58** 304, 1943.

87 Maxon, A. L. *Bulletin* 311, South Dakota Agricultural Experiment Station, 1937.

88 Gyorgy and Goldblatt (footnotes 58 and 59c).

89 Cameron, G. R., and Karunartne, W. E. *J. Path. & Bact.* **42** 1, 1936.

90 Glynn, L. E., and Himsworth, H. P. *J. Path. & Bact.* **56** 297, 1944.

91 Glynn, L. E., Himsworth, H. P., and Neuberger, A. *Brit. J. Exper. Path.* **26** 326, 1945.

92 Handler, P., and Bernheim, F. *J. Biol. Chem.* **148** 649, 1943.

which make methyl groups available for the synthesis of choline. It has also been produced in pancreatectomized dogs maintained with insulin (Chaikoff and co-workers<sup>49</sup>) and is probably due to the impairment of digestion and resulting malabsorption. The condition could be prevented by the administration of lecithin.

**Diffuse Hepatic Cytosiderosis** This has been produced in rabbits by chronic copper poisoning (Mallory and associates<sup>93</sup>, Mallory<sup>94</sup>, Mallory and Parker<sup>95</sup>) and in cats by ligation of the pancreatic duct (Taylor, Stiven and Reid<sup>96</sup>). The condition is interesting, as cytosiderosis is a constant finding in adult pellagrins in South Africa and is frequently associated with cirrhosis of the liver (Gillman and Gillman<sup>97</sup>, Gillman, Mandelstam and Gillman<sup>98</sup>).

**Lobar Hypertrophy With or Without Lobar Absorption, Secondary to Vascular Derangements** This condition has been reported by Gillman and associates<sup>16</sup>.

**Generalized Atrophy** This is one of the categories of diseases of the liver produced by the maize meal diet (Gillman and associates<sup>16</sup>).

**Cirrhosis** Cirrhosis has been produced experimentally by two distinct procedures: (1) repeated administration of various hepatotoxic agents, such as carbon tetrachloride and manganese (Cameron and Karunartne<sup>89</sup>, Moon<sup>99</sup>), and (2) establishing nutritional deficiencies. In the latter case it may be preceded by severe and prolonged fatty infiltration (Chaikoff and associates<sup>49</sup>, Connor<sup>55</sup>, Gyorgy and Goldblatt<sup>58</sup>, Spellberg and Keeton<sup>68</sup>, Blumberg and Grady<sup>51</sup>, Handler and Bernheim<sup>92</sup>, Himsworth and Glynn<sup>100</sup>, Gillman and co-workers<sup>16</sup>) or it may follow postnecrotic scarring with or without nodular hyperplasia (Gyorgy and Goldblatt<sup>88</sup>, Hock and Fink<sup>101</sup>, Himsworth and Glynn<sup>17b</sup>).

An important observation in the pathologic examination of experimental fatty cirrhosis of rats is that of a pigment in the liver (Gyorgy and

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93 Mallory, F. B., Parker, F., Jr., and Nye, R. N. *J. M. Research* **42**: 461, 1921.

94 Mallory, F. B. *Am. J. Path.* **1**: 117, 1925.

95 Mallory, F. B., and Parker, F., Jr. *Am. J. Path.* **7**: 365, 1931.

96 Taylor, J., Stiven, D., and Reid, E. W. *J. Path. & Bact.* **34**: 793, 1931, 41: 397, 1935.

97 Gillman, J., and Gillman, T. *Arch. Path.* **40**: 239, 1945.

98 Gillman, J., Mandelstam, J., and Gillman, T. *S. African J. M. Sc.* **10**: 109, 1945.

99 Moon, V. H. *Arch. Path.* **18**: 381, 1934.

100 Himsworth and Glynn (footnotes 17b and 18).

101 Hock, A., and Fink, H. *Ztschr. f. physiol. Chem.* **279**: 187, 1943.

Goldblatt<sup>59c</sup>, Blumberg and Grady<sup>51</sup>, Edwards and White<sup>102</sup>, Popper, Gyorgy and Goldblatt<sup>103</sup>, Endicott and Lillie<sup>104</sup>, Gyorgy<sup>105</sup>) This pigment has been named "ceroid" by Lillie, Daft and Sebrell<sup>8</sup> Victor and Pappenheimer<sup>106</sup> observed a similar acid-fast pigment in various tissues, including the livers, of certain patients with nutritional disorders and hepatic disease They pointed out the possibility that the pigment was related to vitamin E deficiency Popper, Gyorgy and Goldblatt<sup>103</sup> suggested that ceroid probably arises from fat globules in cells deficient in vitamin E and that its formation is favored by absorption factors and inhibited by lipotropic factors (Pappenheimer and Victor<sup>107</sup>) However, they<sup>103</sup> were not able to demonstrate this pigment in normal or in cirrhotic human livers, nor has it been observed in dietary deficiency cirrhosis in dogs (Chaikoff and co-workers<sup>108</sup>) In human alcoholic cirrhosis, which is not recognized as based on a deficient diet, Mallory<sup>109</sup> described eosinophilic hyaline bodies in the liver cells undergoing degeneration as a characteristic finding These have not been observed in the experimental cirrhosis of rats

From the foregoing review it is apparent that hepatic injury can be produced in animals by various methods and that this injury when severe or prolonged may terminate in cirrhosis It is hardly an exaggeration to say that practically every dietary factor save carbohydrate has, at one time or another, been credited with playing a role in the production of hepatic injury, and the uncertainty in this field is so great that Bollman<sup>110</sup> in a recent review wrote that dietary injury of the liver could apparently be brought about in many different ways

*Summary of Experimental Work*—Two major types of hepatic injury have been produced in animals by feeding them deficient diets The one is fatty infiltration of the liver, which, if pronounced and sustained, may develop into cirrhosis of Laennec's type The other acute necrosis (comparable to acute yellow atrophy of man), which, if not fatal, results in scarring and nodular hyperplasia The two lesions may be present at the same time, and this sometimes makes it difficult to place them in

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102 Edwards, J E, and White, J J Nat Cancer Inst 2 157, 1941

103 Popper, H, Gyorgy, P, and Goldblatt, H Arch Path 37 161, 1944

104 Endicott, K M, and Lillie, R D Am J Path 20 149, 1944

105 Gyorgy, P Am J Clin Path 14 67, 1944

106 Victor, J, and Pappenheimer, A M J Exper Med 82 375, 1945

107 Pappenheimer, A M, and Victor, J Am J Path 22 402, 1946

108 Chaikoff, I L, Eichron, K B, Connor, C L, and Entenman, C Am J Path 19 9, 1943

109 Mallory, F B Bull Johns Hopkins Hosp 22 69, 1911

110 Bollman, J L, in Luck, J M Annual Review of Physiology, Stanford University, Calif, Annual Reviews, Inc, 1943, vol 5, p 321

separate categories. That fatty infiltration may eventually lead to cirrhosis was first observed in depancreatized dogs kept alive with diet and insulin. It was then observed that the cirrhosis could be prevented by feeding the animals lecithin, choline and pancreas. The action of the latter may be due to the lecithin which it contains or to lipocain. The common property of all these substances is their lipotropic activity. On the basis of this theme, an effort was made to produce fatty infiltration by feeding animals a high fat diet. After several months, fatty infiltration and cirrhosis of the portal type developed in these animals. Fatty infiltration acted by causing anoxemia and alterations of cell metabolism, leading to death of liver cells. It was further observed that in the early stages the process was reversible and that if the fat content of the liver could be reduced to normal by the administration of lipotropic substances, e.g., lecithin and choline, the hepatic damage could be prevented from developing into cirrhosis.

Later it was found that while cystine was ineffective, casein or methionine added to the diet prevented fatty infiltration in spite of a high fat content. The action of casein is probably due to one of its component amino acids, methionine. Choline and methionine have in common the methyl group ( $\text{CH}_3$ ) which cystine does not contain. Witts<sup>111</sup> has well illustrated the action of lipotropic agents which centers around the supply of labile methyl groups.

|          |              |           |                  |
|----------|--------------|-----------|------------------|
| Pancreas | → lecithin   | → choline | → } labile       |
| Casein   | → methionine |           | → } methyl group |

When there is deficiency of labile methyl groups, the body is unable to manufacture lecithin, which is required for the transfer of the hepatic fat. The fat accumulates and damages the liver cell by causing anoxemia and alterations of cell metabolism. The ultimate death of cells is followed by avascular fibrosis.

Himsworth and Glynn<sup>17a</sup> showed that protein deficiency, besides causing fatty infiltration, may cause acute damage in the form of necrosis developing within a few weeks. They found that different proteins vary in their ability to prevent necrosis, e.g., casein is effective in small amounts while yeast is ineffective even in large quantities. The main difference between the two proteins is that yeast is poor in sulfur-containing amino acids. The development of the acute necrotic lesions may be prevented if the rats are fed the two sulfur-containing amino acids, methionine and cystine. These observations suggested that the sulfhydryl ( $\text{SH}$ ) group is as essential as the methyl group ( $\text{CH}_3$ ) for the prevention of dietary cirrhosis (Glynn, Himsworth and Neuberger<sup>91</sup>). Methionine, which contains both methyl and sulfhydryl groups, is the key substance in the metabolism of the liver and is one of the most essential amino acids. On

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111 Witts, L. J. Brit. M. J. 1, 1947

the other hand, an excess of cystine may be harmful, and Earle and Victor<sup>83</sup> have produced lesions of the necrotic type by giving 300 mg of cystine per day

The relation of the vitamin B complex to cirrhosis and necrosis has yet to be clearly determined. Some of the B vitamins, particularly biotin, favor the deposition of fat in the rat's liver. On the present evidence it seems justifiable to conclude that the massive dose of yeast or of any other source of the vitamin B complex is not likely to produce ill effects on the liver in hepatic diseases of man (Witts<sup>111</sup>), although the beneficial effects of such a therapeutic regimen are not clearly established.

The effect of diets high in carbohydrate is not clear. It has long been known that in hepatic damage depletion of glycogen takes place and that animals fed a high carbohydrate diet are more protected against drugs which produce fatty infiltration of the liver than are those given a diet high in fat (Rosenfeld<sup>112</sup>). A high carbohydrate diet affords some protection against chloroform poisoning and is of value in the prevention of the hepatic damage following experimental ligation of the common bile duct, the making of Eck's fistula, mushroom poisoning and partial hepatectomy (Soskin and Hyman<sup>113</sup>). The action of carbohydrates is that of fuel for the hepatic cells in their work of transport, protein synthesis and detoxication. The carbohydrates probably act through their sparing of proteins, but they cannot protect the liver unless the requirements of protein metabolism and total calories are satisfied (Witts<sup>111</sup>). If these requirements are not met, an unbalanced carbohydrate diet may actually favor the development of necrosis (Craven<sup>114</sup>).

#### CIRRHOSIS OF THE LIVER IN HUMAN SUBJECTS

*Incidence* —Ratnoff and Patek<sup>115</sup> have given a comprehensive analysis of the world incidence of Laennec's cirrhosis as encountered in autopsy material. They place the highest incidence of cirrhosis in China (12.0 per cent), Zurich, Switzerland (10 per cent), Vizagapatam in South India (9.3 per cent), London, England (7.7 per cent) and Philadelphia (6.1 per cent). In East Africa, Vint<sup>116</sup> reported cirrhosis as observed in 6.7 per cent of the autopsies performed on natives. In the majority of the other countries the incidence varies from 1 to 4.5 per cent.<sup>115</sup>

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112 Rosenfeld, G. *Ergebn d Physiol* 2: 50, 1903.

113 Soskin, S., and Hyman, M. *Arch Int Med* 64: 1265, 1939.

114 Craven, E. B., Jr. *Bull Johns Hopkins Hosp* 48: 131, 1931.

115 Ratnoff, O. D., and Patek, A. J., Jr. *Medicine* 21: 207, 1942.

116 Vint, F. W. *East African M J* 7: 349, 1931.

Cirrhosis is more common in Indians than is usually appreciated, and the high incidence has been constantly recorded by Rogers,<sup>117</sup> Hance,<sup>118</sup> Hughes<sup>119</sup> and Tirmurti and Rao<sup>120</sup> The figures quoted by Ratnoff and Patek<sup>115</sup> for various parts of India are Calcutta, 6.9 per cent, Madras, 4.4 per cent, Vizagapatam, 9.3 per cent

Cirrhosis of the liver is thus encountered in all the countries of the world but is most common in China, South Africa, East Africa, India, Ceylon and Java

*Role of Alcohol and Diet in the Causation of Human Cirrhosis*—It can now be assumed that evidence has accumulated showing that cirrhosis of the liver may have its genesis in some form of nutritional deficiency

**Alcoholism** Alcohol as a direct cause of cirrhosis has been pretty well dismissed (Jolliffe and Jellineke<sup>121</sup>), although when an excessive intake of alcohol is associated with an insufficient diet, fatty infiltration is frequently noted (Connor<sup>56</sup>) In a review of experimental cirrhosis in laboratory animals, Moon<sup>99</sup> cited several workers who failed to produce cirrhosis by feeding animals on alcohol Friedenwald<sup>122</sup> failed to produce cirrhosis in rabbits by administering 5 to 8 cc of absolute alcohol diluted in 20 to 30 cc of water daily over a period of four years

Cirrhosis of the liver is common in India, Java and Ceylon, where alcoholism is rare Tirmurti and Rao,<sup>120</sup> Menon and Annamalai,<sup>123</sup> Hughes<sup>119</sup> and Manson-Bahr<sup>124</sup> concluded, after a detailed survey, that alcohol could not be the cause of cirrhosis in Indians Thus, in Vizagapatam, South India, portal cirrhosis was seen in 5.2 per cent of all the autopsies, but only 3 per cent of the patients with portal cirrhosis had been addicted to the consumption of alcoholic beverages (Sutherland<sup>125</sup>) This opinion has been confirmed by Yenikomshian<sup>126</sup> in Lebanon and Syria, and by Stacey<sup>127</sup> in Iraq

117 Rogers, L (a) Indian M Gaz 46 47, 1911, (b) Lancet 2 355, 1912, (c) Brit M J 1 345, 1922

118 Hance, J B Guy's Hosp Rep 78 379, 1928

119 Hughes, T A Indian J M Research 21 353, 1933

120 Tirmurti, T S, and Rao, M V R Indian M Gaz 69 74, 1934

121 Jolliffe, N, and Jellineke, M Quart J Stud on Alcohol 2 544, 1941

122 Friedenwald, J J A M A 45 780, 1905

123 Menon, T B, and Annamalai, D R Indian J M Research 22 827, 1935

124 Manson-Bahr, P H L Manson's Tropical Diseases A Manual of the Diseases of Warm Climates, ed 12, London, William Wood & Company, 1945

125 Sutherland, D W Indian M Gaz 40 121, 1905

126 Yenikomshian, H A J A M A 103 660, 1934

127 Stacey, R S Tr Roy Soc Trop Med & Hyg 37 387, 1944

Dietary Factors Workers in the East (Yang<sup>128</sup>, Rao<sup>129</sup>, Yenikomshian<sup>126</sup>, Tyagaraja<sup>130</sup>) had suggested that dietary factors were concerned in the causation of hepatic cirrhosis, but diet was never considered of prime importance. Malaria, dysentery and parasitic infections were regarded as directly responsible for the production of cirrhotic livers, with diet playing only a contributory role.

It has long been felt that in Indians cirrhosis of the liver is the result, at least in part, of malnutrition, which is widely prevalent both in acute and chronic forms, owing to poverty and religious restrictions (Hughes<sup>131</sup>, Hughes and Shrivastava<sup>132</sup>). Witts<sup>111</sup> mentioned the differences in the incidence of, and the mortality from, infective hepatitis in the India Command during 1944, from the figures collected by Lt Col Stuart McDonald. The incidence of jaundice was five times as high in the British troops as in the Indian troops, but the mortality was just the reverse. The factor favoring the low incidence of jaundice could be a nutritional difference or a previous exposure, but it is almost certain that the high mortality of Indians with this disease was due to malnutrition.

At the Central Command Conference<sup>133</sup> on anemias in Agra, India, it was definitely brought out by various pathologists that anemia and malnutrition were prevalent in Indian troops. The conditions prevailing among the Indian civilians are similar. The most common type of anemia encountered in Indians with cirrhosis is the normochromic or hypochromic macrocytic type, due primarily to malnutrition and curable by improved nutrition with or without any change in the pathologic condition of the liver.

Nutritional deficiency is also claimed to play a part in the production of cirrhosis in Indian children. Rao<sup>134</sup> reported that cirrhosis is found mostly in the children of vegetarian parents, especially in those infants who are weaned early and who are fed either cow's milk or the family vegetarian diet. It could be prevented by feeding the infants modern patented milks. In a case reported by him, a mother of twins, a boy and a girl, faced with the choice of breast feeding one of them only, weaned the girl and fed her cow's milk. Cirrhosis developed in the girl, while the boy remained healthy.

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128 Yang, C S. *Nat M J China* 14 195, 1928

129 Rao, M V R. *Indian J M Research* 21 389, 1933

130 Tyagaraja, S. *Ceylon J Sc, Sect D, M Sc* (pt 3) 4 119, 1937

131 Hughes, T A. *Indian J M Research* 14 157, 1926. Hughes<sup>119</sup>

132 Hughes, T A, and Shrivastava, D L. *Indian J M Research* 15 427, 1927

133 Proceedings of the Central Command Conference on Anemia, Agra, India, Nov 20-21, 1944

134 Rao, P K. *Proc Indian Acad Sc, Sect B* 14 310, 1941



Gillman<sup>14</sup> reported cirrhosis of the liver to be widely prevalent in the poor natives of the Rand in South Africa, who eat bulky vegetarian diets. They commonly present a condition known as kwashiorkor, characterized clinically by edema, pellagrous lesions of the skin, steatorrhea and microcytic anemia (Trowell and Muwazi<sup>135</sup>). Biopsy or postmortem examination of the liver reveals extreme fatty infiltration (Smith<sup>136</sup>), and there is ample evidence that this is followed by cirrhosis.

During the war the colored troops and the native races of the Middle East and the East showed a high mortality from infective hepatitis. Witts<sup>137</sup> reported an outbreak in the British Colonial troops with a mortality of 40 per cent. In 1943 a high mortality was recorded in a small epidemic among poorly nourished African laborers in Uganda. Of the 14 patients admitted to the hospital, all died (Witts<sup>111</sup>).

In Jamaica, infantile cirrhosis of the liver is common, particularly in the children of unmarried mothers who support themselves and their babies by working. Breast feeding is not too common, the infants are fed mostly on a diet consisting of carbohydrates, arrowroot, cereal, gruel or even sugar and water. It has also been observed that cirrhosis is less frequent in the fishing villages, where the protein content of the diet may be higher (McFarlane and Branday<sup>138</sup>, Platt<sup>139</sup>, Witts<sup>111</sup>).

Foods grown on soil with a high selenium content have been reported to cause severe injury of the liver, which may develop into cirrhosis (Moxon and Rhian<sup>140</sup>). These authors pointed out that persons subsisting on high carbohydrate, low protein diets are more liable to get selenium cirrhosis than those on high protein diets, and concluded that it is the badly balanced diet which prepares the ground for the toxic action of selenium.

*Role of Infection in the Causation of Human Cirrhosis*—Since parasitic infections are so widespread in the countries where cirrhosis is most prevalent, attention has been constantly focused on elucidating the part played by parasitic and other infections in the production of cirrhosis.

The rather frequent background of malaria in Indians has been responsible for constant speculation regarding the possibility that malaria is related to cirrhosis. Sitsen,<sup>141</sup> Hance<sup>118</sup> and Hughes<sup>119</sup> presented ev-

135 Trowell, H. C., and Muwazi, E. M. K. *Arch Dis Childhood* **20** 110, 1945.

136 Smith, E. C. *Tr Roy Soc Trop Med & Hyg* **36** 287, 1943.

137 Witts, L. J. *Brit M J* **1** 739, 1944.

138 McFarlane, A. L., and Branday, W. J. *Brit M J* **1** 838, 1945.

139 Platt, B. S. *Nutrition in British West Indies, Great Britain Colonial* no 195, London, His Majesty's Stationery Office, Oct 9, 1945.

140 Moxon, A. L., and Rhian, M. *Physiol Rev* **23** 305, 1943.

141 Sitsen, A. E. *Geneesk tijdschr v Nederl-Indie* **62** 5, 1921, abstracted, *Trop Dis Bull* **20** 22, 1922.

idence in support of the view that malarial necrosis of the liver is an important cause of cirrhosis Rogers<sup>142</sup> reported that he did not come across malarial cirrhosis in a single one of a series of 5,000 autopsies in Calcutta, where malaria is endemic<sup>143</sup> Tirmurti and Rao<sup>143</sup> reviewed the role of malaria in cirrhosis and studied the livers of malarial subjects They concluded that there was no evidence from pathologic material that malaria produces cirrhosis Osler<sup>144</sup> did not come across a single person with malarial cirrhosis in the wards or the autopsy room at the Johns Hopkins Hospital over a period of fifteen years Stacey<sup>127</sup> found no evidence of malaria either clinically or at postmortem examination in cases of cirrhosis in Iraq ~~—Schistosomiasis (p. 127) is not the cause of cirrhosis~~

Amebiasis leading to amebic hepatitis has been constantly blamed as causing cirrhosis of the liver in the tropics Rogers<sup>117a</sup> propounded the view that the portal cirrhosis of Indians resulted commonly from amebiasis His explanation was that focal and diffuse necrosis of liver tissue caused by amebic infection results in fibrosis Rogers<sup>145</sup> Menon and Annamalai,<sup>123</sup> Rao<sup>129</sup> and Wang<sup>146</sup> reported the incidence of dysentery in patients with cirrhosis in India and China as from 25 to 40 per cent, compared with 7 to 17 per cent in the general population Tirmurti and Rao<sup>147</sup> found no evidence of active or old dysentery in postmortem examinations of 18 persons with cirrhosis of the liver The same authors,<sup>120</sup> discussing the role of amebiasis, argued that the fibrosis occurring around the amebic abscesses was localized and could not be regarded as the cause of diffuse portal fibrosis In examining biopsy specimens of livers from patients with chronic amebic hepatitis at Agra, I never came across a definite appearance of portal cirrhosis, although postnecrotic scarring was not infrequent However, protracted amebiasis with intestinal complications would undoubtedly impair nutrition—a result which would be grave in patients who are already ill fed It could be a precipitating factor in a liver which has already been damaged by malnutrition

Schistosomiasis has been claimed as a cause of cirrhosis in Egypt (Day<sup>148</sup>), where it is endemic However, in India and China (Yang<sup>128</sup>) this type of infection is absent and cannot be considered the cause of cirrhosis In the series reported by Stacey<sup>127</sup> from Iraq, there is no evidence that schistosomal infection is the cause of cirrhosis there

142 Rogers, L Practitioner 131 117, 1933

143 Tirmurti, T S, and Rao, M V R Indian J M Research 24 149, 1936

144 Osler, W The Principles and Practice of Medicine, ed 7, Philadelphia, D Appleton and Company, 1909

145 Rogers (footnotes 117c and 142)

146 Wang, C F Chinese M J 50 891, 1936

147 Tirmurti, T S, and Rao, M V R J Indian M A 1 423, 1932

148 Day, H B Tr Roy Soc Trop Med & Hyg 18 121, 1924

Bacterial and virus infections have been suspected of playing a primary or a precipitating casual role in the production of cirrhosis, especially in children, in whom the illness is usually ushered in by fever. Cirrhosis has been reported occurring in several children of the same family. In all these it usually has been preceded by some acute infection. Ely<sup>149</sup> reported cirrhosis in 4 year old twins whose symptoms began with jaundice which developed during an attack of measles and ended five months later in death. Moon<sup>150</sup> reported cirrhosis occurring in 3 members of one family, following scarlet fever. Hemolytic streptococci were cultivated from the liver of 1 at postmortem examination. The streptococci were inoculated into 12 young rabbits by various routes. The animals lived three to fifteen days, and the hemolytic streptococci were recultured from the livers of 11, the livers showed marked degeneration and necrosis. Moon<sup>150</sup> also cultivated streptococci from the liver in 6 cases of cirrhosis and demonstrated cocci in sections in 9 others in which the cirrhotic process was active. McMahon and Mallory<sup>151</sup> reported that beta hemolytic streptococci were demonstrated in 4 of 5 cases of streptococcic hepatitis.

Livers infected with *Escherichia coli* (McMahon<sup>152</sup>), *Staphylococcus aureus* (Phillipps<sup>153</sup>) or *Treponema pallidum* (McMichael<sup>154</sup>) have also been reported to show a similar picture. Rao<sup>134</sup> reported that *Bacillus coli* had been cultured from liver in 1 of his cases. Harrell and McBryde<sup>155</sup> in a recent review concluded that dietary deficiency combined with repeated infection is an important etiologic factor of the cirrhosis developing in children. In most of these the condition was probably hepatic necrosis followed by postnecrotic scarring and nodular hyperplasia.

*Clinical Application of Experimental Observations*—The medical literature of recent years contains a large number of reports of cases in which disease of the human liver was treated with specific diets. These cases can be grouped into two major categories: cases of chronic hepatic cirrhosis and cases of acute infectious and toxic hepatitis.

Patek<sup>156</sup> observed that in cases of cirrhosis associated with alcoholism high vitamin therapy led to improvement of hepatic function. Lewis,

149 Ely, T. Boston M. & S. J. 170 542, 1914

150 Moon, V. H. Am. J. M. Sc. 177 681, 1929

151 McMahon, H. E., and Mallory, F. B. Am. J. Path. 7 299, 1931

152 McMahon, H. E. Am. J. Path. 7 77, 1931

153 Phillipps, F. A. Lancet I 1050, 1937

154 McMichael, J. J. Path. & Bact. 39 481, 1934

155 Harrell, G. T., and McBryde, A. Am. J. Dis. Child. 59 1301, 1940

156 Patek, A. J., Jr. Proc. Soc. Exper. Biol. & Med. 37 329, 1937

Taylor and Davidson<sup>157</sup> obtained satisfactory results in 19 patients with portal cirrhosis by the use of a "liver protein digest ("ledinac")"—a granular powder prepared by vacuum drying of a partial enzymatic (papain) hydrolysate of the beef liver pulp remaining after hot aqueous extraction of the antipericious anemia factor. The preparation contains 60 per cent protein in addition to large quantities of the known components of the vitamin B complex.

Working on the experimental evidence that the absence of lipotropic amino acids produces fatty infiltration of the liver, Fagin and Zinn<sup>158</sup> reported encouraging results in patients treated parenterally with amino acids. Franklin and co-workers<sup>159</sup> studied the histologic changes in the livers by repeated biopsies before and after institution of "lipotropic therapy" (cystine plus choline, methionine and high protein, high vitamin B complex diet). They found disappearance of fat and regeneration of parenchymal cells in all human fatty cirrhotic and noncirrhotic livers. This change was associated with clinical and functional improvement. However, they could not find any evidence of arrest of the cirrhotic process after the removal of fat. This disappearing of fat without concomitant decrease of fibrous tissue after lipotropic therapy has also been reported by Gillman and Gillman<sup>160</sup> and Beams and Endicott<sup>161</sup>. These observations are in accord with clinical findings of Weir<sup>162</sup> and Beams<sup>163</sup>.

Morrison<sup>164</sup> adopted the therapeutic regimen of a high protein, low fat, moderate carbohydrate diet, frequent feedings of skimmed milk and methionine, choline chloride, and a special liver extract containing the vitamin B complex, with favorable results. In recent years various other workers<sup>165</sup> have reported that patients with cirrhosis of the liver improve

157 Lewis, J. H., Taylor, F. H. L., and Davidson, C. S. *New England J Med* 236 351, 1947

158 Fagin, I. D., and Zinn, F. T. *J Lab & Clin Med* 27 1400, 1942

159 Franklin, M., Salk, M. R., Steigmann, F., and Popper, H. *Am J Clin Path* 18 273, 1948

160 Gillman, T., and Gillman, J. *Arch Int Med* 76 63, 1945

161 Beams, A. J., and Endicott, E. T., cited by Franklin, M., Salk, M. R., Steigmann, F., and Popper, H. *Am J Clin Path* 18 273, 1948

162 Weir, J. *J A M A* 134 579, 1947

163 Beams, A. J. *J A M A* 130 190, 1946

164 Morrison, L. M. *J A M A* 134 673, 1947

165 (a) Patek, A. J., Jr., and Post, J. *J Clin Investigation* 20 481, 1941 (b) Fleming, R. G., and Snell, A. M. *Am J Digest Dis* 9 115, 1942 (c) Broun, G. O., and Muether, R. O. *J A M A* 118 1403, 1942 (d) MacKenzie, C. G., and Seligson, D. *Federation Proc* 1 187, 1942 (e) Daft, F. S., Sebrell, W. H., and Lillie, R. D. *Proc Soc Exper Biol & Med* 50 1, 1942 (f) Hoagland, C. L. *New York State J Med* 143 1041, 1943 (g) Russaloff, A. H., and Blumberg, H. *Ann Int Med* 21 848, 1944 (h) Rimmerman, A. B.,

or recover after receiving diets rich in protein or in one or the other of its component amino acids, especially choline and cystine, or methionine

Lately the trend has been to use various combinations of diets high in protein with a high carbohydrate and a moderate to low fat content, plus fortified casein hydrolysate, dried brewers' yeast orally, liver extract parenterally, and specific vitamins and amino acids when these are indicated (Patek<sup>156</sup>, Patek and Post<sup>155a</sup>, Fleming and Snell<sup>155b</sup>, Butt and Snell<sup>156</sup>, Brown and Muether<sup>155c</sup>, Hoagland<sup>155f</sup>, Jolliffe and Alpert<sup>157</sup>, Morrison<sup>155k</sup>, Lewis and co-workers<sup>157</sup>, Wade<sup>158</sup>) The most promising results were observed in cases of enlarged liver, in which the enlargement was probably due to fatty infiltration, which disappeared after the use of lipotropic agents. Once fibrosis develops, it is hardly possible to effect any improvement, though recently Sellers<sup>159</sup> has shown in rats an apparent decrease in fibrosis after lipotropic activity

Patients have been treated for infectious hepatitis with methionine, with fairly good results (Beattie and Marshall<sup>170</sup>, Eddy<sup>171</sup>) On the other hand, Wilson and associates<sup>172</sup> and Higgins and Associates<sup>173</sup> have reported that the results obtained with methionine in epidemic hepatitis were disappointing. Following the suggestion of Glynn, Himsworth and Neuberger<sup>91</sup> that cystine was more potent in preventing hepatic necrosis, Wilson, Pollock and Harris<sup>174</sup> treated patients for infectious hepatitis with cystine and found a significant decrease in the relapse rate

Results with the use of methionine in toxicopathic hepatitis have been reported by several investigators—in carbon tetrachloride poisoning by Eddy<sup>175</sup> and Beattie and co-workers,<sup>176</sup> in hepatitis due to trinitrotoluene

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Schwartz, S. O., Popper, H., and Steigman, F. *Am J Digest Dis* **11** 401, 1944  
 (s) Barker, W. H. *M Clin North America* **29** 273, 1945 (j) Ralli, E. P., Robson, J. S., Clark, D. H., and Hoagland, C. L. *J Clin Investigation* **24** 316, 1945 (k) Morrison, L. M. *Ann Int Med* **24** 465, 1946 (l) Patek, A. J., Jr. *New York State J Med* **46** 2519, 1946 (m) *J Mt Sinai Hosp* **14** 1, 1947

166 Butt, H. R., and Snell, A. M. *Proc Staff Meet, Mayo Clin* **17** 250, 1942

167 Jolliffe, N., and Alpert, E. *M Clin North America* **29** 655, 1945

168 Wade, L. J. *M Clin North America* **29** 479, 1945

169 Sellers, E. A. *Federation Proc* **6** 290, 1947

170 Beattie, J., and Marshall, J. (a) *Nature, London* **153** 525, 1944, (b) **154** 547, 1944

171 Eddy, J. H. *Am J M Sc* **210** 374, 1945

172 Wilson, C., Pollock, M. R., and Harris, A. D. *Brit M J* **1** 399, 1945

173 Higgins, G., O'Brien, J. R. P., Peters, R. A., Stewart, A., and Witts, L. *J Brit M J* **1** 401, 1945

174 Wilson, C., Pollock, M. R., and Harris, A. D. *Lancet* **1** 881, 1946

175 Eddy, J. H. *J A M A* **128** 994, 1945

176 Beattie, J., Herbert, P. H., Wechtel, C., and Steele, C. W. *Brit M J* **1** 209, 1944

by Eddy<sup>171</sup> and in jaundice complicating arsphenamine therapy by Eddy<sup>171</sup> and in jaundice complicating arsphenamine therapy by Beattie and Marshall<sup>170a</sup> and by Peters and associates<sup>177</sup>

( Although animal experiments have clarified the probable etiologic roles of various factors of cirrhosis of the liver, there are many obscure and unexplained things in the genesis of cirrhosis and other hepatic lesions which must be considered before applying the results of animal experiments to man. The most significant contribution of the dietary experiments on animals has been the appreciation of the effect of diet in modifying the degree to which the liver reacts to various hepatotoxic agents. That injury of the liver can be produced by dietetic means is an accepted fact, but the factors responsible are still a source of debate. Every dietetic factor, at one time or another, has been implicated. One must remember, when applying the results to clinical usage, that most of the experiments in animals at present are conducted with synthetic diets which are highly purified and consequently decidedly different from the natural diets of the natives among whom cirrhosis is common. ) From this point of view, Gillman<sup>174</sup> made the first major contribution toward the understanding of the relation between diet and human cirrhosis. He investigated the effect on rats of prolonged feeding of the staple diet (mealie pap) of the South African natives and found that nodular cirrhosis developed in the rats.

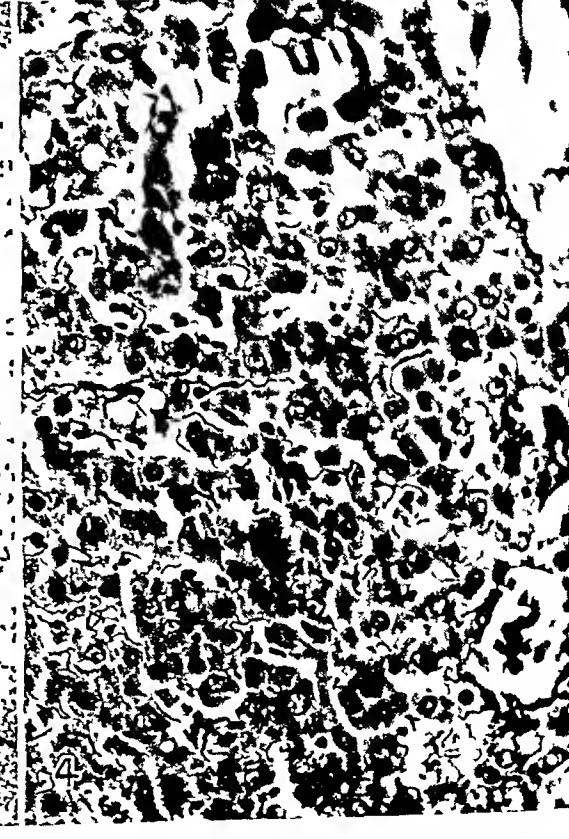
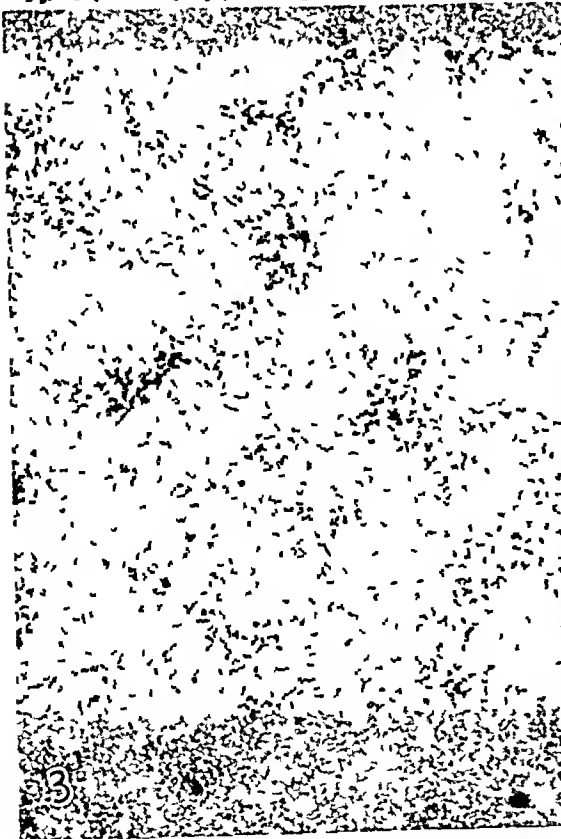
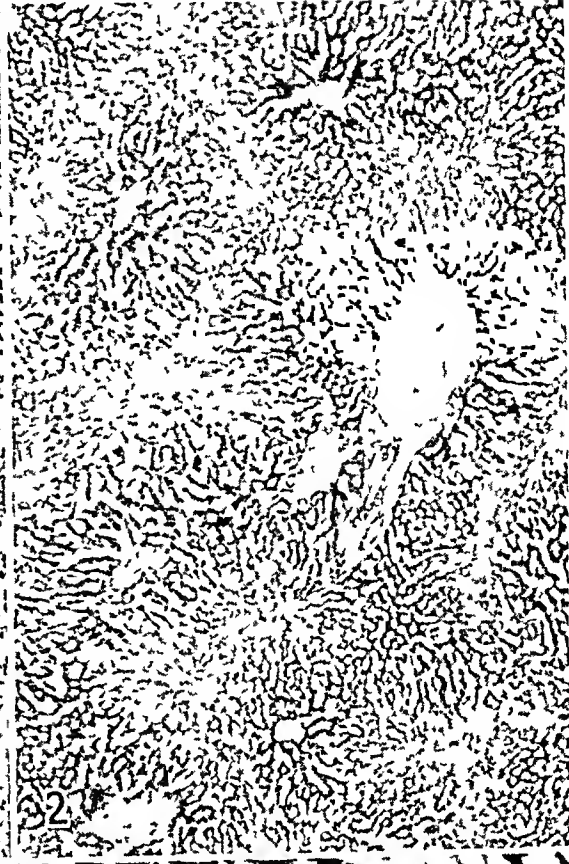
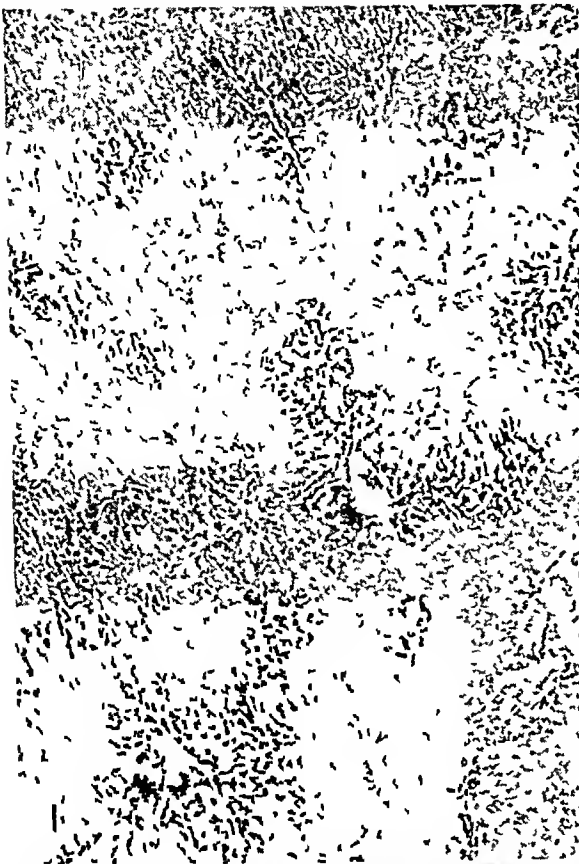
Many are inclined to attribute the high incidence of cirrhosis in Indians to a racial or a genetic factor. They refuse to consider environment as enhancing the sensitivity of a race to the factors productive of hepatic injury. This opinion is based on the fact that cirrhosis of the liver is found particularly in the pigmented people, e.g., Javanese, Indians, Chinese, Japanese and South Africans. It must, however, be remembered that another feature common to these, besides the pigment, is the poverty which compels them to subsist on an unbalanced diet composed of limited amounts of the cheapest foods. However, it is also conceivable that in these countries infections like malaria, amebiasis and schistosomiasis may be responsible for giving the final blow to an already impoverished liver.

#### OBJECTIVES OF THE PRESENT RESEARCH

This paper represents a preliminary report on one aspect of a comprehensive program initiated at the New England Deaconess Hospital, Boston, in which the effects of various diets on laboratory animals are being investigated with a view to determining the exact role played by diet in the production of cirrhosis of the liver in Indian adults and in-

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<sup>177</sup> Peters, R. A., Thompson, R. H. S., King, A. J., Williams, D. I., and Nicol, C. S. *Nature*, London 153: 773, 1944.



phants This work will be continued in the pathology laboratory of the Agra Medical College In subsequent papers the cirrhosis of the liver found in Indians will be discussed and compared with the experimental cirrhosis of rats from the points of view of pathology and histology

### METHODS AND MATERIALS

*Animals*—Two sets of albino "Wistar" rats were used in the present experiment One set consisted of newly weaned rats weighing approximately 50 Gm These were taken to study the effect of deficient diets on infant livers, as infantile cirrhosis is common in India The second set of rats weighed approximately 100 to 150 Gm at the beginning of the experiment All the animals in one experimental group were of the same sex and approximate weight The older ones were kept in single cages, while the infant rats were grouped according to the types of diet they were receiving Once a week the animals were weighed, the older ones individually and the younger ones in groups In the case of the latter the average weight of the animals was determined

*Feeding*—The animals were fed at the same time each day The older rats were given 8 Gm of food each, and the younger ones 5 Gm, irrespective of the nature of the diet Their consumption of water was not restricted Before each feeding the residue of the previous meal was collected and weighed As the diets were moistened with water before administration, allowance was made for water when the amount of food eaten was calculated

*Diets*—The diets, which were similar to the Himsworth-Glynn<sup>17b</sup> carbohydrate diet, were divided into two groups, one containing 7 per cent yeast and the other 4 per cent casein The high carbohydrate, low protein, low fat diets are the nearest to the diets of vegetarian Hindus, among whom most cases of cirrhosis occur

The ingredients of the diets included

Carbohydrates Crushed maize starch was used

Fat Lard was used as the source of fat

Yeast Brewer's yeast no 1 of the Vitamin Food Company was used The powder contained protein 49.7 per cent, fat 2.8 per cent, carbohydrate 36.7 per cent, minerals 7.6 per cent and moisture 3.2 per cent

Casein Glaxo Laboratories grade A casein was used

Salt mixture All the diets contained a salt mixture (3 per cent) made up of potassium iodide 1 per cent, calcium phosphate 1 per cent and sodium chloride 98 per cent

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Fig 1—Section of rat's liver showing areas of necrosis Evidence of post-necrotic scarring is not seen microscopically, but quantitative determination revealed an increase of fibrous tissue Hematoxylin and eosin,  $\times 50$

Fig 2—Section of rat's liver showing distended sinusoids and flattened liver cells Hematoxylin and eosin,  $\times 50$

Fig 3—Section of rat's liver showing extensive fatty infiltration Hematoxylin and eosin,  $\times 50$

Fig 4—Section of rat's liver stained for reticulin fibers Condensation and thickening of reticulum can be seen The fibrous tissue was quantitatively determined as 0.9 per cent of the net weight of the liver Foot's silver stain,  $\times 325$



**Vitamins** All the diets contained cod liver oil (1 per cent) A daily supplement of water-soluble vitamins was given to each animal as follows—thiamine hydrochloride 20 mg, riboflavin 20 mg, pyridoxine 10 mg, calcium pantothenate 100 mg and nicotinamide 50 mg These were added to the food just before it was given to the animals

TABLE 1—*Composition of the Two Types of Diet*

| Carbohydrate-Yeast-Protein Diet | Per Cent | Carbohydrate-Casein-Protein Diet | Per Cent |
|---------------------------------|----------|----------------------------------|----------|
| Salt mixture                    | 3        | Salt mixture                     | 3        |
| Cod liver oil                   | 1        | Cod liver oil                    | 1        |
| Lard                            | 5        | Lard                             | 5        |
| Maize starch                    | 84       | Maize starch                     | 87       |
| Yeast protein                   | 7        | Casein                           | 4        |
| Vitamin supplement              |          | Vitamin supplement               |          |

**Histologic Examination**—Liver samples were fixed in formaldehyde-saline and Zenker-acetic acid solutions Pieces of liver were taken from the middle of the left and the middle of the right lobe, and from any other part which aroused a suspicion of pathologic change Sections were stained with hematoxylin and eosin and with phloxine-methylene blue Besides these, sections from each paraffin block were stained with Masson's trichrome stain, Van Gieson's stain for fibrous tissue and Foot's silver stain for reticulum fibers Frozen sections were stained for fat with scarlet red

**Quantitative Estimation of Fibrous Tissue**—The quantitative determination of the fibrous tissue in normal and pathologic livers of rats was done by the method of Lowry and associates<sup>178</sup> This method has been of great help in detecting an increase of fibrous tissue in livers in which histologic examinations did not reveal definite microscopic changes Working with human livers, Warren and Wahi<sup>179</sup> found that the chemical determination is a more accurate method of detecting the presence and the extent of fibrosis in liver than the histologic examination

**Estimation of Hepatic Fat**—This was carried out in accordance with the method described by Leathers and Raper<sup>180</sup>

## RESULTS

The behavior of the animals on carbohydrate diets provided some interesting observations In the beginning, all the animals seemed to thrive, taking interest in the surroundings, eating well and gaining weight after an initial fall Then seemingly normal animals would suddenly fall ill and succumb, or would rally only to relapse again after a few weeks

178 Lowry, O H, Gilligan, D R, and Katersky, E M J Biol Chem 139 795, 1941

179 Warren, S, and Wahi, P N Arch Path 44 563, 1947

180 Leathers, J B, and Raper, H S The "Fats," London, Longmans, Green & Company, 1925

After one or a number of such attacks, all the animals died. The first indication of their illness was a loss of appetite. The animal would be huddled in a corner of the cage, and the food remained untouched. If the illness was severe but not fatal, the rat remained ill, ate little and continued to lose weight. If it survived this period, or if the illness was not acute, it became more lively and began eating, and its weight became stationary. However, after a second or a third severe attack, it would succumb.

Infant rats succumbed earlier, and all were dead in forty to seventy-seven days. Adult rats showed better resistance, the first one dying after one hundred days. After this they died at various intervals corresponding to the various episodes of illness. Rats on microscopic examination showing changes leading ultimately to fibrosis had an almost uneventful clinical course. They retained their activity and appetite to the end. The only indication of something wrong was an erratic weight curve showing rapid falls and equally rapid recoveries. If not killed, the animal usually died of some indefinite illness. Fatty infiltration of the liver was seen in the animal which died on the hundredth day, but early cirrhotic changes were present only in animals living longer.

#### PATHOLOGIC CHANGES

*Livers of Rats Weighing 50 Gm*—The infant rats showed changes which were different in many respects from those observed in the adults. Neither showed gross or microscopic changes characteristic of portal cirrhosis (diffuse hepatic fibrosis) as described by Himsworth and Glynn<sup>17b</sup>. On microscopic examination, the liver was swollen and reddish purple, and petechiae were present. One rat which survived a few weeks after a severe attack of illness had a reddish yellow liver which was fairly smooth. It also had ascites. In none of the infant rats were advanced lesions of nodular hyperplasia observed as reported by Himsworth and Glynn<sup>17b</sup>. Probably these rats did not live long enough for fibrosis to develop to the point of causing severe distortion and nodularity of the liver. Microscopically, areas of liver with parenchymatous cells completely dead were seen alternating with areas of normal liver parenchyma (fig. 1). In places the necrosed cells could be seen being phagocytosed by polymorphonuclear leukocytes, although areas of necrosis without any leukocyte reaction were not uncommon in such livers. Fatty infiltration of the liver cells was not seen to any impressive degree. In the livers of the animals which had survived the initial illness a noninflammatory fibroblastic reaction was noted in the necrotic areas. Although the special stains did not show any appreciable increase of fibrous tissue, the quantitative determination revealed an increase of collagen ranging from 0.5 to 0.8 per cent.

Another lesion encountered was the enormous dilatation of sinusoids (fig 2) with no definite areas of necrosis or fibrosis. The liver cells bordering the dilated sinuses were flattened and had a finely granular and often vacuolated cytoplasm which did not stain for fat. Some cells around the portal tracts contained fat droplets, which were mostly small and numerous, although an occasional large globule could be seen occupying the whole cell and pushing the nucleus to one side. Special stains did not reveal an increase of reticulum but showed the reticulum condensed along the liver cell cords.

*Livers of Rats Weighing 100 Gm or More*—The changes observed in the livers of the older animals were different. These livers showed heavy fatty infiltration, represented mostly by single large fat globules, in almost all the liver cells (fig 3). The sinusoids associated with the distended cells were almost obliterated. At this and later stages, staining with phloxine-methylene blue did not reveal Mallory's hyaline bodies, supposed to be characteristic of alcoholic cirrhosis. In animals living longer the condensing of reticulum fibers along the liver cell cords was seen. This was followed by the stretching of the fine reticulum fibers that circumscribe the lobules. The liver cells at the periphery of the lobules showed degenerative changes. The cytoplasm was granular and vacuolated, and the nuclei were pyknotic. These vacuolated cells have been described by Mallory<sup>109</sup> and Connor<sup>55a</sup> as characteristic of active cirrhosis.

Among those rats maintained for a longer time on the diet an increasing condensation of reticulum occurred around the lobules as well as inside the lobules along the liver cell cords (fig 4). Fibrous tissue was being laid down along the large portal tracts. In some regions the bile ducts appeared proliferated and dilated. At this stage there was a definite decrease in the amount of fat in the liver cells, and whatever there was occurred in the form of small discrete droplets. None of the livers with outstanding fatty infiltration showed any necrotic areas, although predominantly necrotic changes, associated with minimal fatty infiltration, similar to changes in the livers of the younger rats, were also seen in some adult animals.

In the early stages no microscopic evidence of hepatic damage was seen. The sections stained with the trichrome stain did not reveal definite fibrosis. The stain for reticulum showed increasing condensation of the fibers. The most reliable method was the quantitative determination of fibrous tissue by the method of Lowry and associates<sup>178</sup>. The average normal quantity ranged up to 0.4 per cent of the wet weight of the liver, as determined in 8 normal rats. Histologically, none of these showed increased reticulum. In early cases in which microscopic examination showed only condensation and increase of the reticulum, the quantitative

determination gave figures up to 0.9 per cent. In only 1 rat, in which the amount of fibrous tissue was 1.2 per cent, did the microscopic examination give definite evidence of early cirrhosis in the form of fibroblastic activity and increased periportal fibrosis.

Table 2 shows the amounts of fibrous tissue in pathologic livers.

The adult rats thus showed hepatic changes comparable to early portal cirrhosis of man, though a few revealed only focal necrosis. None had the advanced cirrhosis characterized by irregularity of surface externally and fibrosis and disorganization of the lobular pattern microscopically. Only 1 rat had ascites, which was minimal, and none showed jaundice. There was no definite line of demarcation between the types of pathologic change produced by the casein-deficient and the yeast-deficient diet except that the animals restricted to the latter diet showed lesions earlier.

TABLE 2—*Correlation of Microscopic and Chemical Evidence of Fibrosis in Rats' Livers*

| Rat | Microscopic Observation                                   | Fibrous Tissue, Per Cent<br>of Wet Weight of Liver |
|-----|---|--|
| 1   | Increased reticulum? - - -                                | 0.5  |
| 2   | Increased reticulum - - -                                 | 0.8  |
| 3   | Increased reticulum - - -                                 | 0.75   |
| 4   | Increased reticulum - - -                                 | 0.9  |
| 5   | Increased reticulum - - -                                 | 0.85   |
| 6   | Increased reticulum and increased periportal fibrosis - - | 1.2  |

### COMMENT

The lesions seen in the rats on the high carbohydrate, low protein diet in the present experiment are different in some respects from the changes reported by other workers, especially Himsworth and Glynn<sup>17b</sup> and Gillman and associates<sup>16</sup>. The diet used was the synthetic diet nearest to the natural high carbohydrate and low protein meals eaten by orthodox Hindus, whose staple diet is wheat, rice or maize. The children also eat the same foods in the form of gruel or porridge.

Changes produced in infant were different from those produced in adult rats. In the former the outstanding lesion was focal or diffuse necrosis followed by noninflammatory fibroblastic activity. That acute necrosis is followed by fibrosis was shown by the increase of fibrous tissue as determined by quantitative estimation. This finding is consistent with the changes Himsworth and Glynn<sup>17b</sup> observed in the livers of rats subsisting on their high carbohydrate, protein-deficient diets except that none of my rats showed extensive scarring and macroscopic nodular hyperplasia. It may be that the rats in the present series did not survive long enough for extensive fibrosis to develop. None of the livers showed fatty changes.

Himsworth and Glynn<sup>17b</sup> made a similar observation and stated that "fatty infiltration of liver is not a necessary antecedent of massive necrosis, but may, on the contrary, serve as a protection against its development."

Some of the infant rats also showed dilatation of sinusoids, with associated flattening of liver cells, and diffuse enlargement of the lobule, but none of them showed atrophy or disappearance of the affected lobules as reported by Gillman and co-workers<sup>16</sup>. Hemorrhage was also absent, though at times sinusoidal distention with cellular atrophy was mistaken for it.

In the adult rats the liver cells' accumulation of fat seemed to be the obvious reaction to the high carbohydrate, low protein diet. In some of the animals patches of focal necrosis developed similar to those in the infant rats, the same animals had only minimal fatty infiltration. This is identical with the observation of Himsworth and Glynn<sup>17b</sup> that hepatic necrosis developed with minimal fatty infiltration in rats fed protein-deficient diet.

However, a good percentage showed extensive fatty infiltration of the liver, almost all the cells being filled with single large fat droplets. The livers of these rats resembled those reported by Gyorgy and Goldblatt,<sup>181</sup> Chaikoff, Connor and Biskind,<sup>49</sup> Blumberg and Grady<sup>51</sup> and Gillman and associates<sup>16</sup>. Variations were seen in the further development of the disease process from this stage onward. Some of the livers did not show any increase of reticulum either microscopically or chemically. However, they showed an increase of weight due to fatty infiltration, and when the amount of fibrous tissue determined quantitatively was calculated relative to the normal weight of livers, these livers could be classified as precirrhotic (Warren and Wahl<sup>179</sup>). Other livers showed microscopic signs of incipient cirrhosis—decrease of fat, condensation and thickening of perilobular and intralobular reticulum, increase of periportal connective tissue with small round cell infiltration, dilatation and proliferation of bile ducts, and quantitative increase of fibrous tissue content. The type of cirrhosis observed by Blumberg and Grady,<sup>51</sup> Chaikoff and associates,<sup>50</sup> and Connor<sup>55a</sup> in depancreatized dogs, by Connor<sup>55b</sup> in human beings suffering from alcoholism and in patients with diabetes and by Gillman and associates<sup>16</sup> in rats was preceded by fatty infiltration of liver cells of long standing, but there was no hemorrhage or necrosis. Himsworth and Glynn<sup>17b</sup> have recently reported that diffuse hepatic fibrosis developed in rats restricted to a high fat diet. This was preceded by fatty infiltration of the liver cells. In the presence of severe protein deficiency they observed the development of hemorrhagic necrosis affecting the entire liver. If the animal succumbed, they felt that this was similar to the acute yellow atrophy observed in man, if it survived, it showed

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181 Gyorgy and Goldblatt (footnotes 59a and c)

nodular hyperplasia of the liver comparable to the healed yellow atrophy in man

The left lobe of the liver was found to be more involved than the right, and in certain cases the pathologic change was confined to the left lobe. This confirms the similar observations of Himsworth and Glynn<sup>17b</sup>. Their explanation for it seems the most acceptable. They ascribed it to the fact that in the portal vein the blood coming from the superior mesenteric vein tends to the right and so passes up the right branch of the portal vein to the right lobe of the liver. The blood from the splenic and inferior mesenteric veins keeps mainly to the left and reaches the left lobe of the liver. This difference has been demonstrated also in dogs on injection of emulsified fat (Bartlett and co-workers<sup>182</sup>), india ink (Copher and Dick<sup>183</sup>) and radioactive phosphorus (Hahn, Donald and Grier<sup>184</sup>). When the material was injected into the superior mesenteric vein, it was carried to the right lobe, and when it was injected into the inferior mesenteric or the splenic vein, it was carried to the left lobe. A similar tendency has been noted by Gillman<sup>14</sup>.

Thus the right lobe of the liver is supplied through the superior mesenteric vein with blood predominantly from the small intestine and receives thereby the products of the latter's digestion, which contain great amounts of those constituents of protein required to prevent massive necrosis. When the supply of these essential constituents is insufficient, the right lobe gets most of it and escapes necrotic changes. In the left lobe, receiving blood from the large bowel, which is poor in protein constituents, necrosis develops.

From the available evidence there seems to be little doubt that fatty livers can be produced by deficient diets, whether these contain a high percentage of fat or not. In the present series, as well as in those reported by Gillman and associates,<sup>16</sup> high carbohydrate, protein deficient diets have produced extensive fatty infiltration. Such livers may undergo necrosis and postnecrotic scarring, or they may undergo cirrhosis. Gyorgy and Goldblatt<sup>58</sup> made a careful study of the possibility that exogenous or endogenous toxins may cause the hepatic damage observed in their animals. They observed that damage of the liver occurred only under specific dietetic conditions and came to the conclusion that if toxins were responsible they exerted their effects only on animals having a particular dietetic background. Their animals first showed diffuse fatty changes of the liver, and thereafter some other factor, either exogenous or en-

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182 Bartlett, F. K., Corpcr, H. J., and Long, E. R. *Am. J. Physiol.* **35**: 36, 1914.

183 Copher, G. H., and Dick, B. M. *Arch. Surg.* **17**: 408, 1928.

184 Hahn, P. F., Donald, W. D., and Grier, R. C., Jr. *Am. J. Physiol.* **143**: 105, 1945.

dogenous, induced hemorrhagic necrosis and finally a condition which they considered cirrhosis but which probably was postnecrotic scarring and nodular hyperplasia. In the experiments described by Gillman and associates,<sup>16</sup> the livers became diffusely fatty, and thereafter there was great diversity in the sequence of events in the development of the pathologic process in the different lobes of the same liver.

In the present series both these changes were seen either individually or concomitantly in rats fed a diet high in carbohydrate and low in protein. The obvious indication is that with the same diet and under the same conditions the animals may get focal or diffuse necrosis with or without postnecrotic scarring, fatty infiltration alone or followed by incipient cirrhosis, and, finally, a combination of postnecrotic scarring and portal cirrhosis. What is it, then, which decides what type of lesion develops? Is it the length of time which the animal survives on the deficient diet? As Himsworth and Glynn<sup>17b</sup> noted, "the animals least adroit in obtaining food would die of necrosis, those more adroit would survive to develop diffuse fibrosis." However, this does not seem to be the whole story, for on the basis of the present experiments, as well as on the basis of the work of Gillman and associates,<sup>16</sup> it can be said that fatty liver is an essential precursor of cirrhosis, although even when of long standing the fatty infiltration does not necessarily terminate in cirrhosis. This leads one to the conclusion that the injury of the liver cells implied in fatty infiltration does not necessarily lead to fibroblastic reaction. This is in accordance with the observations of Gillman and associates,<sup>16</sup> who noted the disappearance of the whole of the trabeculae and the lobules without any evidence of fibrous tissue reaction and concluded that nodular hyperplasia and cirrhosis are manifestations of stimuli exciting proliferative activity of both the liver cells and the connective tissue.

Mallory<sup>109</sup> also pointed out that fibroblasts do not proliferate when liver cells alone are destroyed. Lucke<sup>185</sup> reported similar pathologic observations in regard to epidemic hepatitis, in which there was no formation of collagen though there was extensive destruction of parenchyma. The independence of the hepatic parenchyma and the connective tissue with respect to injury was also noted by Daft, Sebrell and Lillie.<sup>165e</sup> The question again arises as to the role of a possible toxin, endogenous or exogenous, acting on a protein-depleted liver. The absence of lipotropic amino acids leads to fatty infiltration, but the proliferation of fibrous tissue requires an independent stimulus. Is the proliferation of fibroblasts in alcoholic cirrhosis due, as Mallory<sup>109</sup> suggested, to injury caused

mechanically by cells of exudative origin stretching the connective tissue, or is it due to a toxin whose nature is yet to be determined?

It is interesting to compare these experimental observations with those on the livers of infants with cirrhosis in India, where the histologic development of cirrhosis is more on an infectious basis. Broad bands of fibrous tissue, patches of necrosis, cellular infiltration, complete disorganization of the liver pattern and absence of fatty changes are outstanding in the microscopic observations. The changes are more of the nature of postnecrotic scarring leading ultimately to nodular hyperplasia, rather than portal cirrhosis. Clinically, infantile cirrhosis in India is often a familial disease. The illness is usually ushered in by fever and other symptoms of acute infection. These facts make one wonder whether infection of some sort does not play a precipitating role, especially in the light of Rao's<sup>134</sup> report that he obtained a culture of *B. coli* in one of his cases. He described polymorphonuclear infiltration of the periportal areas as a constant finding on microscopic examination. It may be that in the familial instances the cause is a virus infecting the mother's genital tract, the infant contracting the infection during birth. This suggestion is along the lines of recent work of Budding<sup>186</sup> in which he isolated a virus identical with the virus of infantile diarrhea from the adult female genital tract. He observed that "the probability that the virus may be acquired, transmitted, and maintained venereally in adults and thus serve as the original source of epidemics of diarrhea of the new-born, must be entertained." Nevertheless, the absence of protective proteins in the undernourished vegetarian infant plays an important role in that it prepares the liver to be finally knocked out by a precipitating factor like infection.

#### SUMMARY AND CONCLUSIONS

Rats weighing approximately 50 Gm and fed a diet high in carbohydrate and low in protein show hepatic damage, which expresses itself as necrosis of the parenchyma. This may end in postnecrotic scarring if the animal lives long enough.

Animals weighing over 100 Gm are more resistant to the effect of this deficient diet and live longer. Their livers show varieties of lesions like focal or diffuse necrosis, fatty infiltration and incipient cirrhosis.

In the development of cirrhosis of the liver there are two stages, the first consists of diffuse fatty infiltration of the liver cells, and the second, of fibrous tissue proliferation and regeneration of liver cells.

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185 Lucke, B. *Am. J. Path.* 20: 471, 1944.

186 Budding, G. J. *South. M. J.* 39: 382, 1946.



Cirrhosis of the liver is not a necessary sequel to injury of the liver cells, and fatty infiltration does not necessarily terminate in cirrhosis

It is possible that some noxious agent like a toxin, bacterium or a virus, especially in infantile cirrhosis, acts as a precipitating factor

The quantitative determination of fibrous tissue has been found more useful in determining the presence of cirrhotic changes in early stages than the microscopic examination of stained sections

# CONGENITAL MALFORMATION OF VERTEBRAE (HEMIVERTEBRAE) WITH APLASIA OF CORRESPONDING RIBS, ASSOCIATED WITH A LATERAL MENINGOMYELOCELE

A Report of a Case

I RALPH GOLDMAN, M D  
LOS ANGELES

**C**ONGENITAL absence of one or more ribs accompanied or not accompanied by hemivertebrae is relatively rare. When, in addition, such a congenital defect is accompanied by a lateral meningomyelocele, it is truly a rarity. These defects may occur with or without other congenital anomalies.

Several papers<sup>1</sup> published before the advent of roentgenography reported cases of absence of one or more ribs in which the diagnosis was made purely on physical examination. Three cases,<sup>2</sup> however, had the benefit of autopsy. The first roentgenographically proved case of congenital absence of a rib was reported in 1899 by Freund.<sup>3</sup> In 1944 Cohn<sup>4</sup> reviewed all the cases of congenital absence of one or more ribs in the literature and classified them into three groups: (1) those in which one rib was missing unilaterally or bilaterally<sup>5</sup>, (2) those wherein

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From the Department of Laboratories, Division of Pathology, Sinai Hospital, Baltimore

1 (a) Lallemand, 1826, cited by Kienbock<sup>6b</sup> (b) Packard, J. H. *Proc Path Soc Philadelphia* 2: 121, 1867 (c) Gage, H. *Tr Am Orthop A* 2: 233, 1889 (d) Sabrazès *Rev de med* 14: 1010, 1894 (e) Thomson, J. *Teratologia* 2: 1, 1895 (f) Murray *Tr Clin Soc London* 29: 252, 1896, cited by Smith<sup>6e</sup>

2 Ardouin, P., and Kirrmisson, E. *Rev d'orthop* 8: 104, 1897. Gripat, H. *Bull Soc anat de Paris* 47: 124, 1874, cited by Smith<sup>6e</sup>. Vrolich, cited by Kienbock<sup>6b</sup>

3 Freund, W. *Jahrb f Kinderh* 49: 349, 1899

4 Cohn, B. N. E. *Am J Roentgenol* 52: 494, 1944

5 (a) Allen, I. *Canad M A J* 28: 69, 1933 (b) Anderson, W. W., and Cathcart, D. F. *Arch Pediat* 49: 827, 1932 (c) Fabris, S. *Pediatrics* 34: 1310, 1926 (d) Gladstone, R. J. *J Anat & Physiol* 46: 220, 1911 (e) Hadda, S. *Ztschr f orthop Chir* 31: 176, 1913 (f) Hatch, H. S., and Plume, C. A. *J A M A* 99: 1254, 1932 (g) Lieberknecht, A. *Beitr z klin Chir* 51: 89, 1906 (h) Pels-Leusden. *Chir Kongr-Verhandl* 72: 1911, cited by Hadda<sup>5e</sup> (i) Putti, V. *Fortschr a d Geb d Rontgenstrahlen* 14: 285, 1909, 15: 65 and 243, 1910 (j) Sever, J. W. *Boston M & S J* 186: 799, 1922

two or more consecutive ribs were lacking on the same side<sup>6</sup>, (3) a miscellaneous group which does not fit either of the foregoing classifications.<sup>7</sup> He presented cases which fitted each classification and pointed out that congenital absence of a rib is commonly associated with presence of a hemivertebra.

An intrathoracic meningocele presenting a round shadow in roentgenograms was reported by Pohl<sup>8</sup> in 1934. In 1940 Ameuille, Willmoth and Kudelski<sup>9</sup> reported finding an intrathoracic mass in a 48 year old patient who complained of thoracic pain. A surgical procedure revealed the mass to be a lateral meningocele with intrapleural development arising from a lateral defect in the eighth and ninth thoracic vertebrae. Arnt<sup>10</sup> reported a simple case of hemivertebrae in 1946.

#### REPORT OF CASE

The patient was a full term white boy who died the second day following delivery. There was no familial history of congenital defects, nor was there a history of contagious diseases occurring during the mother's pregnancy. Examination revealed a white boy with a chest grossly deformed as a result of absence of the first through the ninth right ribs. The tenth, eleventh and twelfth right ribs appeared to be fused posteriorly. In the right posterior wall of the chest there was a large orange-shaped mass which was soft, cystic and could be transilluminated. A thoracic scoliosis to the left was present. There was a clubbed right foot, also a congenital dislocation of the right hip.

*Autopsy*—Postmortem examination revealed complete absence of the right halves of the first through the ninth thoracic vertebrae and absence of their transverse processes. The corresponding right ribs were also absent. The tenth, eleventh and twelfth right ribs were present but were fused posteriorly. Extending laterally to the right from the spinal canal through a defect in the neural arches of the first through the ninth thoracic vertebrae was a pedicle 2.0 by 1.5 cm. in size, which was continuous with the meninges. The pedicle also included a portion of spinal cord. Attached laterally to this pedicle was a large, cystic structure measuring 6.0 by 5.5 by 5.0 cm. and containing a clear fluid. It possessed no communication with either the arachnoid or the subdural spaces. However, a communicating channel was present in the pedicle between the cystic structure and the central canal of the spinal cord just superior to the pedicle. It barely admitted a probe. The central canal of the spinal cord superior to this lumen was greatly dilated, measuring 1.0 cm. in diameter. The surrounding neural tissue was greatly thinned.

6 (a) Erkes, F. *Deutsche Ztschr. f. Chir.* 114: 239, 1912. (b) Kienbock, R. *Fortschr. a. d. Geb. d. Röntgenstrahlen* 13: 269, 1909. (c) Rees, H. L. *Lancet* 1: 916, 1930. (d) Riether, G. *Wien. klin. Wchnschr.* 23: 306, 1910. (e) Smith, C. *J. A. M. A.* 60: 895, 1913. (f) Steel, W. D. *Brit. M. J.* 2: 16, 1939. (g) Hadda<sup>5e</sup>. (h) Putti<sup>5i</sup>.

7 Gotzky, F., and Weihe, F. *Fortschr. a. d. Geb. d. Röntgenstrahlen* 21: 408, 1914. Joachimstahl, G. *Ztschr. f. orthop. chir.* 25: 18, 1910. Putti<sup>5i</sup>. Sever<sup>5j</sup>.

8 Pohl, R. *Röntgenpraxis* 5: 747, 1934.

9 Ameuille, P., Willmoth, P., and Kudelski, C. *Bull. et mém. Soc. méd. d. hôp. de Paris* 56: 608, 1940.

10 Arnt, F. *Röntgenpraxis* 14: 384, 1942.

out and measured only 0.1 to 0.2 cm in thickness. The cystic mass was covered on its anterior surface by parietal pleura (figs 1 and 2). A clubfoot was present on the right. The head of the left femur was displaced posteriorly and inferiorly to a position beneath the gluteal muscles. The right lung was smaller than the left and had no middle lobe. A small azygos lobe measuring 0.7 by 0.4 by 0.3 cm was present on the anterior medial aspect of the upper lobe of the right lung. The heart revealed a widely patent foramen ovale measuring 0.5 cm in diameter.



Fig 1—Roentgenogram showing congenital hemivertebrae of the first through the ninth thoracic vertebrae associated with aplasia of the corresponding right ribs. Note the fusion of the tenth, eleventh and twelfth right ribs.

The ductus Botalli was widely patent. The right renal artery, renal vein, kidney and ureter were absent. The left kidney was of average size and shape and weighed 15 Gm. Its pyramids were somewhat blunted, and the corresponding calices were ballooned out. The pelvis was somewhat dilated. The left ureter was markedly dilated and tortuous. It measured 1.3 cm in circumference. Its wall was thickened. Its ureterovesical orifice was widely patent. The right testicle was present in the abdominal cavity, the left was present in the inguinal canal. The left adrenal gland was of normal size and shape and weighed 2.8 Gm. The right adrenal gland was less than half the size of the left and weighed only 1.0 Gm.

## COMMENT

Hemivertebra and the associated aplasia of the corresponding rib can be explained on an embryologic basis. From the ventromesial face of each somite a group of mesenchymal cells arise which collectively are called a sclerotome. These mesenchymal cells migrate from each side toward the midline, becoming aggregated about the notochord. It is from these masses of cells that the elements of the vertebral column and the ribs arise.



Fig 2—Dissection of the thorax showing the absence of the right half of the first through the ninth vertebrae and their corresponding right ribs. *M* indicates the meningocele, *P*, the pedicle, *S*, the syringocoele.

The first significant change that occurs in these primordial masses is the clustering of sclerotomal cells, derived in part from each of two adjacent somites, into groups which are located opposite the intervals between the myotomes. Each of these cell clusters forms the primordium of the centrum of a vertebra. Once formed, they rapidly become more dense and definitely circumscribed (fig 3). Shortly after the formation of the centrum, paired mesenchymal concentrations extended dorsally and laterally from the centrum becoming the primordia of the neural arches and ribs (fig 4).

The blastemal stage is the stage in which the earliest parts of the skeleton become recognizable and is rapidly followed by the cartilage

stage The conversion to cartilage begins in the blastemal masses, first in the region of the centrum, following which centers appear in each neural arch and costal process These centers spread rapidly and fuse

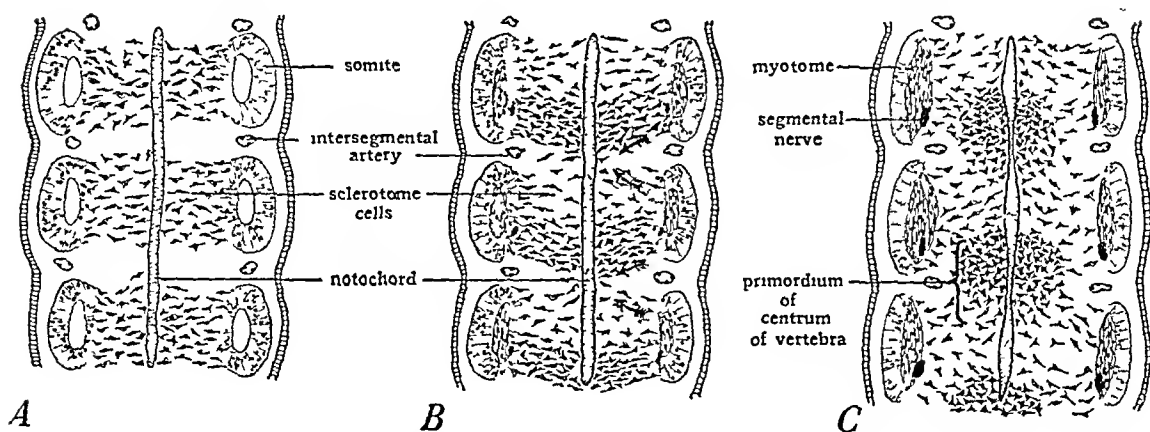


Fig 3—Mesenchymal cells arising from the ventromesial faces of the somites (in A), migrating toward the midline represented by the notochord (in B) and becoming aggregated about the notochord (in C) Each cell cluster in C forms the primordium of the centrum of a vertebra (The illustrations shown in figures 3, 4 and 5 are taken from Bradley M Patten's book "Human Embryology," published in 1946 by the Blakiston Company, Philadelphia)

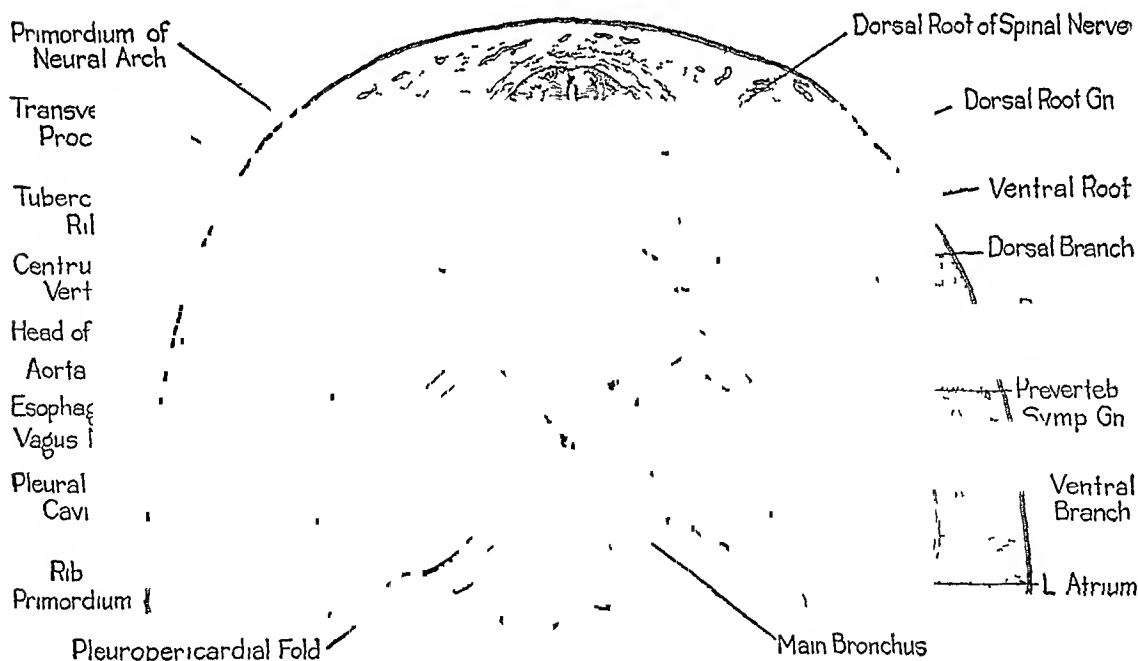


Fig 4—Primordia of the neural arch and transverse process of the vertebra and primordia of the rib—all being developed out of the centrum of the vertebra

until the entire mass is involved The cartilaginous miniature of the vertebra thus formed is first a single piece showing no lines of demarcation where the original centers of cartilage formation became confluent

Neither does it reveal the separate parts of which it will consist after the cartilage has been replaced by bone. By the time ossification begins, the rib cartilages have become separated from the vertebrae themselves, remaining in one piece.

The locations of the endochondrial ossification centers appearing in a vertebral cartilage are shown in figure 5. The median ossification center forms the centrum. The centers in the neural processes extend dorsally forming laminae that complete the neural arch. A prolongation of these same centers beyond their point of junction, dorsal to the centrum, forms

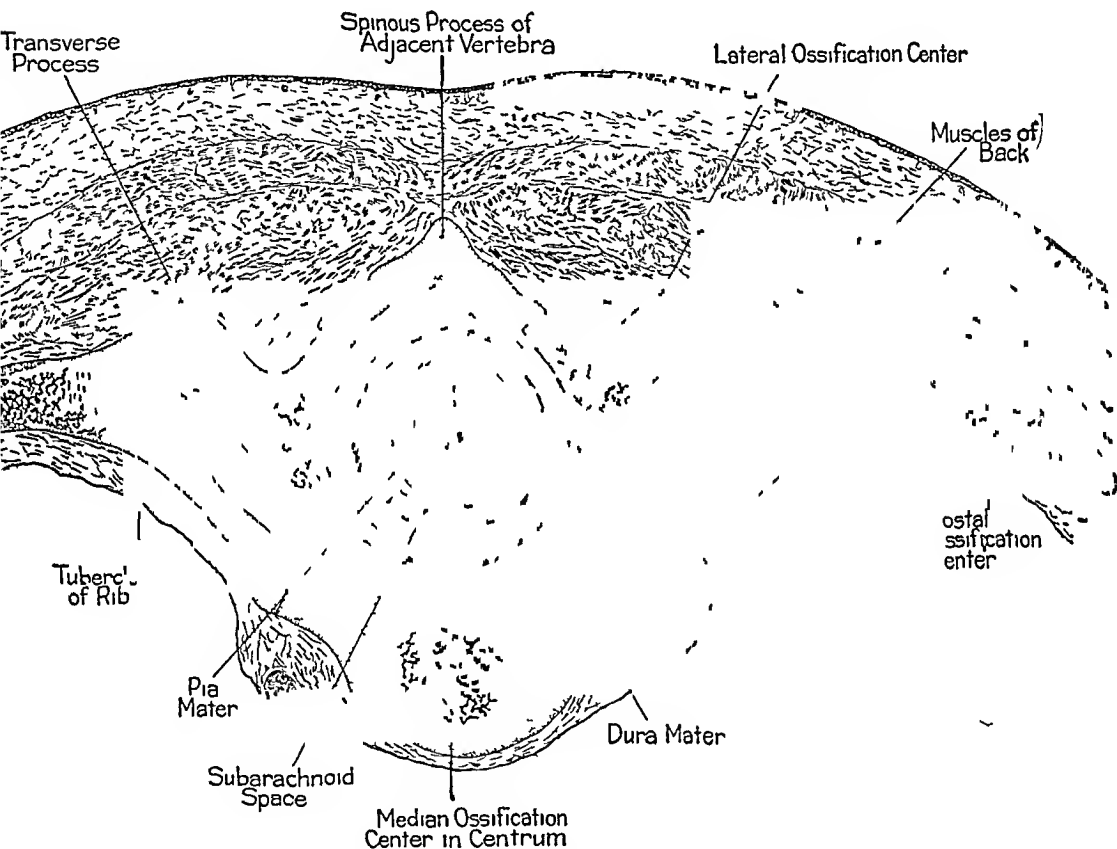


Fig 5—Further development of the vertebra, with centers of ossification indicated

the spinous process. Lateral extensions of the centers appearing in the neural processes form the transverse processes with which the ribs articulate. The neural processes also extend ventrally fusing with those in the centra.

The shafts of the ribs are formed by extensions of their primary ossification centers. Secondary epiphysal centers appear in the tubercle and in the head of the rib after birth. During the period of growth they remain separated from the shafts of the ribs with persistence of cartilage.

plates The secondary epiphysial centers fuse with the shaft when the skeleton acquires its adult dimensions <sup>11</sup>

Thus the relationship between the primordium of a rib and the corresponding vertebra is developed from the embryologic point of view It is seen, therefore, that anything interfering with the development and growth of the primordium of a vertebra may simultaneously be reflected in the growth and development of the transverse process, the rib, the neural arch and even the spinous process—consequently, the frequently associated aplasia of a rib and hemivertebra

Early writers felt that defects of the development of ribs were due to pressure of an arm against the thoracic cage secondary to a lack of amniotic fluid <sup>12</sup> They felt that the arm occupied a hernial defect in the thoracic wall However, the relationship between aplasia of a rib and hemivertebra tends to rule out this theory Stockard<sup>13</sup> in 1921, as a result of his work on the developmental changes occurring in the periods of both determinate and indeterminate cleavage resulting from outside influences, such as changes in hydrogen ion concentration, moisture, oxygen supply and temperature, in the eggs of *Fundulus*, postulated a more widely accepted theory He felt that in the development of every organ or part there is a critical stage characterized by rapid cell multiplication during which it is dominant over nearby organs If during this critical period an environmental change acts adversely on the dominant organ, it may lose its dominance to another organ, this results in an imperfectly formed organ Thus not only must the organ originate from a definite primordium, but it must arise at the appropriate time if it is to assume its normal development and growth This theory appears most tenable in explaining a congenital hemivertebra and the associated aplasia of the corresponding rib Simultaneously the adverse stimulus responsible for the formation of a hemivertebra may act on other developing organs during their critical period to produce concomitant congenital defects The defect of the neural arches present in this case allowed the meninges to herniate into the posterior wall of the chest with resultant formation of a meningomyelocele

#### SUMMARY

A case of a lateral meningomyelocele with absence of the right halves of the first through the ninth thoracic vertebrae and the associated ribs is presented, together with roentgenograms and autopsy observations The embryonal development of the vertebral body and its corresponding ribs is discussed, as is the theory of the genesis of these defects

11 Patten, B M Human Embryology, Philadelphia, The Blakiston Company, 1946

12 Rutter and Eppinger, cited by Hadda <sup>5e</sup> Kienbock <sup>6b</sup> Thomson <sup>1d</sup>

13 Stockard, C R Am J Anat 28 115, 1921



## EXPERIMENTAL LIPOSARCOMA

### Characteristics of Growth Under Low and Under High Caloric Intake

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THE PERTINENT problems of fat tissue new growth are the same as those which obscure the embryogenesis of normal fat tissue. A chemical difference of the lipoma fat and the fat of the normal fat depots has been proposed but no difference of sufficient magnitude has been revealed by chemical methods. An analysis of lipoma fat by Jaeckle<sup>1</sup> showed a remarkably close resemblance to normal subcutaneous human fat. The fatty masses of adiposis dolorosa were also found to be quite similar to normal fat by Edsall.<sup>1</sup> Failing adequate explanation on a structural or a chemical basis, Wells<sup>2</sup> has suggested that tumor fat tissue differs from normal subcutaneous fat because of deficiency or abnormality of the enzymes of fat metabolism. This theory was supported by the conclusion of Kastle and Leavenhart<sup>1</sup> that fat storage is dependent on the presence of the enzyme lipase, which acts reversibly either to split fat into fatty acids and glycerin or to synthesize fat from these diffusible constituents as they are provided by the blood. The absence of lipase in the tumor fat might explain its inavailability to the host fat tissue, it does not explain, however, how storage of fat occurs in the tumor.

Nerve supply and hormone control have been given consideration. Boeke's<sup>3</sup> demonstration that isolated sympathetic nerve fibers are present in individual cells and that denervated fat tissue deposits contain more fat than normal deposits has some bearing on the conception that abnormal nerve control may lead to abnormal storage and metabolism of fat. The observation of Beznak and Hasch<sup>4</sup> that there is an increase in weight of the fat deposits on the sympathectomy

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1 Cited by Wells<sup>2</sup>

2 Wells, G. Arch Int Med 10 297, 1912

3 Boeke, J. Ztschr f mikr-anat Forsch 33 233, 1933

4 Beznak, A. B., and Hasch, Z. Quart J Exper Physiol 27 1, 1937

mized side and that fat persists longer at the site of intervention in emaciation further emphasizes the influence of the sympathetic supply on trophism and growth of fat tissue. The same is true of hormone control, so much emphasized that any further mention would be repetition. Along this line is Geschickter's report<sup>5</sup> that xantholipomatous proliferations were produced in subcutaneous tissue by injecting chorionic gonadotropin extracted from pregnancy urine.

Although the evidence at hand is not sufficient to allow the conclusion that caloric restriction may be used to affect tumor growth, controlled experiments have shown that caloric intake as well as the percentage and the composition of ingested fat and of certain members of the vitamin B complex can influence carcinogenesis (King<sup>6</sup>). If this is the case for all tumor growths, regardless of their origin and structure, as seems likely, it must be more true for the fat tissue new growths, since they arise from cellular elements constantly subject to a variety of complex chemical processes, synthesis, interconversion, degradation, all comprehensively indicated by the expression "molecular regeneration."

#### PURPOSE OF INVESTIGATION

The main point of the present investigation was to study the influence of caloric restriction, on one side, and of a high caloric intake, on the other, in the development and progress of an experimentally produced lipid growth.

Although conclusions as to normal cell origin cannot properly be drawn from the study of rapidly multiplying tumor cells, still it was thought that by following the patterns of growth closely some valuable information might be obtained about the growth of normal and cancerous fat tissue.

#### MATERIAL AND METHODS

White mice of identical breed, male and female indifferently, from 6 to 8 weeks old, and weighing from 12 to 15 Gm, were used as experimental animals. As carcinogenic agent 1, 2, 5, 6-dibenzanthracene was used. The basic diet fed to the animals consisted of the usual mice biscuits containing not less than 14.50 per cent crude protein, 2.05 per cent crude fat, 18 per cent crude fiber and 44 per cent nitrogen-free extract. Ingredients were oats, corn meal, soy bean oil meal, corn germ meal, alfalfa meal, wheat gray middlings, molasses, riboflavin supplement, 1.5 per cent calcium carbonate and 0.5 per cent iodized salt. The group of animals whose caloric intake was to be restricted received daily one third of the ration given the control group. To the basic diet of the group of animals that were to receive high caloric diet a slice of bacon was added daily.

A striking loss of weight in the animals of the first group and an equally striking increase of weight in the animals of the second group, little affected by

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5 Geschickter, C. F. *Am. J. Cancer* **21** 617, 1934.

6 King, C. G. *Ann. New York Acad. Sc.* **44** 3, 1947.

the development of the tumor, were evidence of the effects of the diets. This was further shown at the postmortem examinations by the depleted aspects of the fat depots in one group of animals and by their repleted appearance in the other group.

As it was felt that the composition (melting point, length of fatty acid chains) and therefore the rate of absorption of the fatty solvent carrying the carcinogenic hydrocarbon might have some bearing on the incidence and progress of the tumor, for some of the animals the carcinogen to be injected was dissolved in corn oil, and for some other animals the same carcinogen was dissolved in emulsified pork fat.

With all groups of animals a 5 per cent solution of the carcinogen was used, 0.3 cc of the compound was injected into the subcutaneous fat of each axillary fossa, each inguinal region and the interscapular gland.<sup>7</sup> This distribution was suggested by the known abundance of fat tissue in these locations.

Over the entire experimental period, which lasted nine months, all the animals were systematically examined every six days. Some of the animals were killed at the first appearance of the tumor, others were killed after increasing intervals, and in still others the tumor was allowed to follow its natural course.

The 48 mice used in the experiment were divided into the following four groups: (1) animals receiving 1, 2, 5, 6-dibenzanthracene in corn oil solution—low caloric intake (14 animals), (2) those receiving 1, 2, 5, 6-dibenzanthracene in corn oil solution—high caloric intake (14 animals), (3) those receiving 1, 2, 5, 6-dibenzanthracene in pork fat solution—standard diet (10 animals), and (4) those receiving 1, 2, 5, 6-dibenzanthracene in corn oil solution—standard diet (10 animals). Group 4 was the control group.

## RESULTS

*Structural Characteristics of the Produced New Growth*—Regardless of the difference of caloric intakes and of vehicles of the carcinogen, the gross and the structural characteristics of the growth were invariably the same in the different groups of animals. The gross appearance of each tumor was that of a poorly defined mass, moderately firm in consistency. The color varied from area to area, with an alternating of yellowish gray and grayish pink areas, which found an explanation in the complexity of the cellular patterns as revealed by the microscopic study. In no instance was evidence found that the tumor had metastasized to any of the internal organs.

Besides the structural characteristics, the marked tendency of the tumor to infiltrate locally and the successful transplanting of three different tumors into two additional series, each consisting of 2 animals, clearly showed the aggressive tendencies of the growth. 0.5 cc of a suspension of the tumor (1 to 5 in saline solution) injected deeply into the subcutaneous tissue of the groin was productive in all but 1 of a fairly

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<sup>7</sup> The interscapular gland is a peculiar mass of tissue composed of an interlacing of vascular lymphoid tissue and of fat cells. A number of students have accredited to it a lipid storage function corresponding to that of hibernating glands of insectivora and bats.

rapidly growing tumor that exactly reproduced both grossly and microscopically the characteristics of the original tumor. Recession of the growth did not take place in any case, and in a few instances the tumor

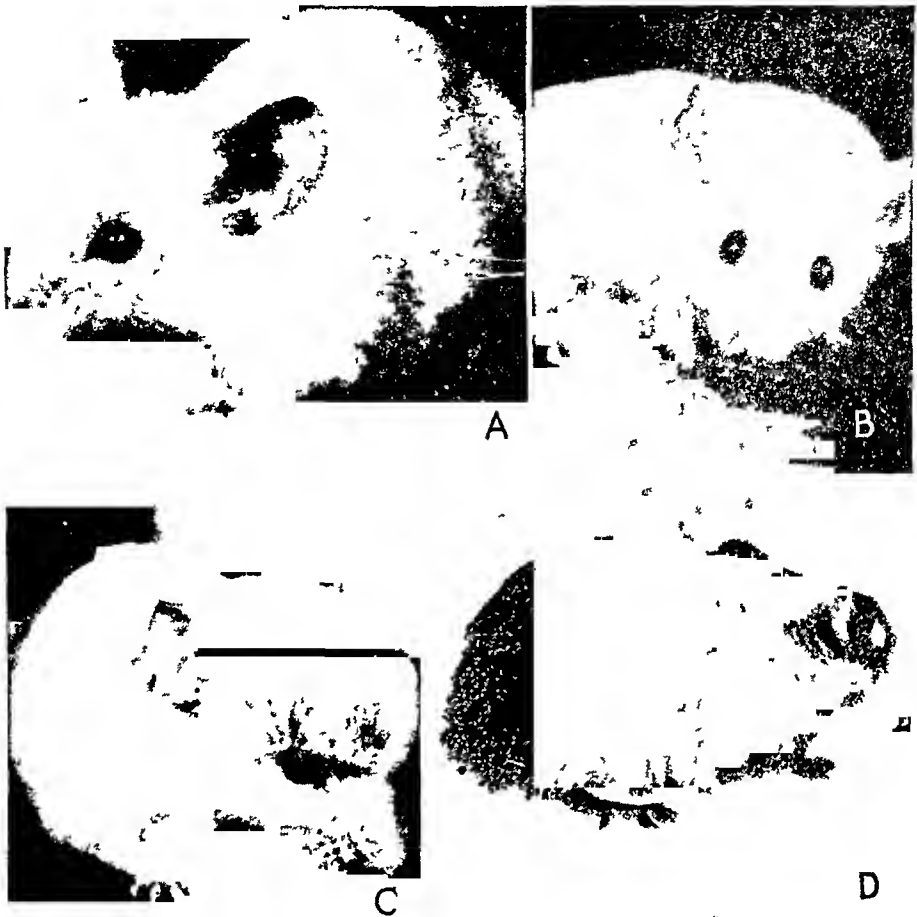


Fig 1—*A*, mouse 45, one hundred and forty days after injection of 1, 2, 5, 6-dibenzanthracene, displaying a mass at the left axillary region. The mouse was given the carcinogen in corn oil with the standard diet.

*B*, mouse 35, one hundred and thirty-eight days after injection of 1, 2, 5, 6-dibenzanthracene, showing two large new growths, one at the right inguinal region and the other at the right axilla. The mouse received carcinogen in pork fat with the standard diet.

*C*, mouse 3, one hundred and fifty days after injection of 1, 2, 5, 6-dibenzanthracene, revealing a large mass in the interscapular region extending over the scalp and forehead. The mouse was given the carcinogen in corn oil with a low caloric diet.

*D*, mouse 16, one hundred and forty-five days after injection of 1, 2, 5, 6-dibenzanthracene, showing tumor involvement of the right axillary and inguinal regions. The mouse was given the carcinogen in corn oil with a high caloric diet.

showed a tendency to ulcerate at the surface, however, in order to detect the progressive developmental phases of the growth, many animals were killed before the tumor had reached full development, and therefore it

cannot be ruled out that this complication might have occurred more frequently if the animals had survived longer

The structure of the tumor consisted mainly of a loose-textured framework of well vascularized fibrous tissue in which a variety of cells was contained, widely spaced in some areas and closely packed in others. The most common cell type was represented by spindle cells, thickly interlaced. They resembled basically the cells of medullary fibrosarcoma, but the cellular endings tended to be blunt instead of pointed, and the cytoplasm was more acidophilic than in the ordinary fibrosarcoma cell. The nuclei were often hyperchromatic and the nucleoli prominent. Mitosis was at the rate of 1 per high power field. Staining for fat droplets revealed none in these spindle cells. The intercellular substance had a few delicate interlaced argentaffin fibers in the meshes of which was contained an amorphous eosinophilic material which could not be stained with mucicarmine.

Fat cells, either sparse or grouped together in alveolar arrangement, were present in this fibrosarcomatous tissue. In some areas the lipid structures prevailed, in some others the fibrosarcomatous elements were predominant. The fat cells ranged in shape from oval to round or polyhedral, and closely resembled the steatoblast or embryonal fat cell of rodents. In the adult fat cells the nuclei occupied the usual peripheral position, in the more immature fat cells the nuclei were either peripherally or more centrally placed. Some of the nuclei were small and vesicular, others large and deeply stained, occupying from one third to one half the diameter of the cell. An occasional nucleus contained a single large nucleolar structure, almost as large as the entire nucleus. The cellular cytoplasm had a foamy appearance and contained a delicate network of granules which enclosed fat globules. The latter ranged from small and dustlike to large granules occupying a good portion of the cellular cytoplasm.

In the midst of these fat cells, irregularly stellate cells, often fused together in syncytial masses, and very large cells containing bizarre-shaped nuclei were not uncommon. These large cells varied in size depending on the degree to which the eosinophilic and finely granular cytoplasm was distended by fat globules. At first glance these giant cells seemed to contain several nuclei, however, on more careful examination each cell was found to possess a single nucleus with a varied number of lobes connected by narrow bands of nuclear substance. In their intimate cytologic characteristics these large cells closely resembled the small granular immature fat cells, and often transitional patterns could be recognized between the two cell types.

Another element in the growth that deserves special mention was a round cell which in many respects resembled a large plasma cell. The

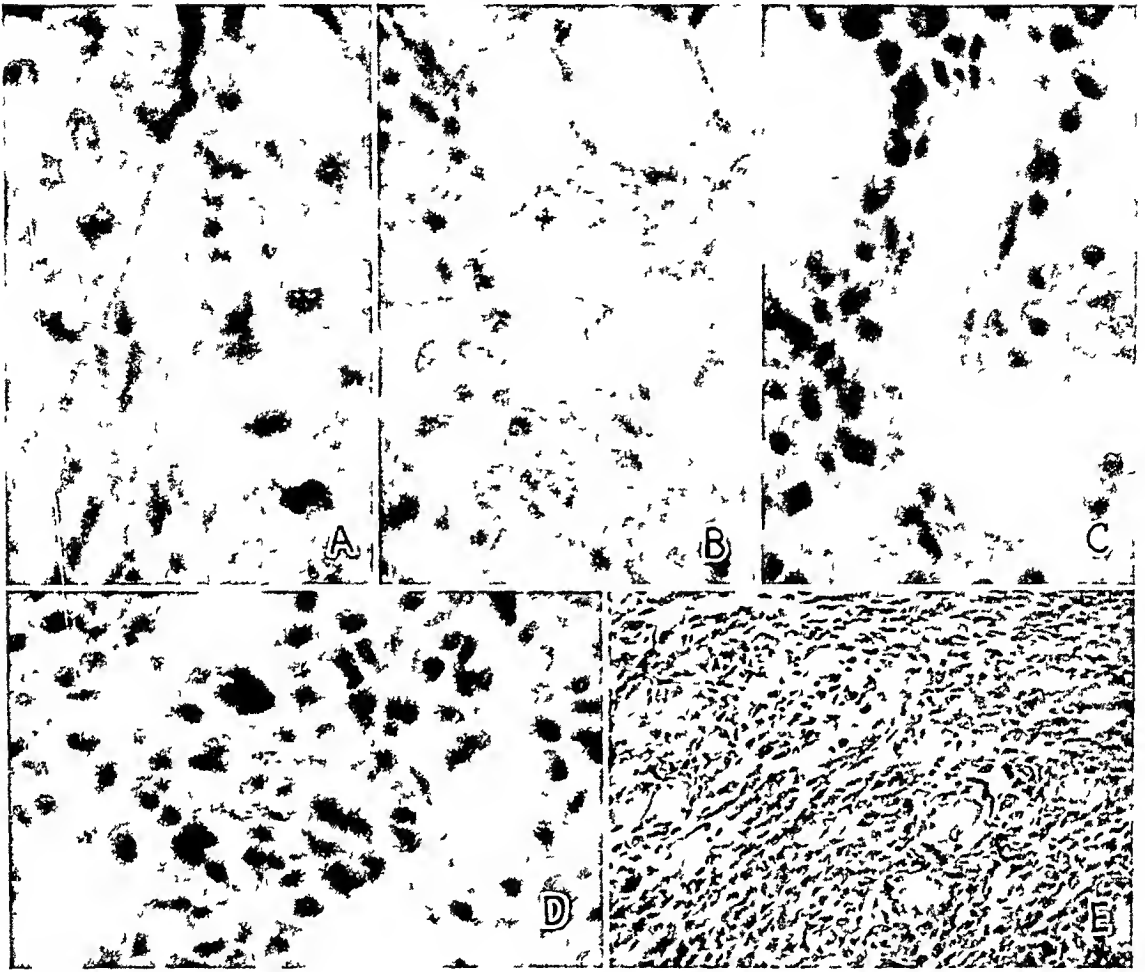


Fig 2—A, early histiocytic mobilization in the fat tissue septums. The tissue was removed from a nodule 0.3 cm across in the right inguinal region of a mouse ninety days after injection of the carcinogen. This mouse belonged to group 1, fed a low caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

B, pleomorphic proliferation of mesenchymal cells in the fat tissue septums. The great variability of cellular forms exhibited is regarded as an expression of the multiple developmental potentialities of the "dormant" undifferentiated mesenchymal cells of the fat lobule which under carcinogenic stimulation differentiated into a variety of connective tissue cells. The tissue was removed from a nodule 0.5 cm across in the left axillary region of a mouse ninety-eight days after injection of the carcinogen. This mouse belonged to group 2, fed a high caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

C, diffuse infiltration of the fat tissue septums by lymphocytoid cells and by cells resembling large plasma cells. Cells with two nuclei are present. The tissue was taken from a nodule 0.4 cm across in the left inguinal region of a mouse one hundred and three days after injection of the carcinogen. The mouse belonged to group 4, fed the standard diet. Photomicrograph, ocular 5, objective 40, Zeiss.

D, predominance, in the growth, of cells resembling plasma cells with patterns suggesting transitional stages in the development of lipoblastic cells. The tissue was removed from a nodule 0.3 cm across in the right axillary region of a mouse one hundred and nine days after injection of the carcinogen. The mouse belonged to group 2, fed a high caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

E, thickly interlaced spindle cells, resembling basically the cells of medullary fibrosarcoma, infiltrating the muscle bundles of the thigh. The tissue was taken from a full grown tumor of the right groin, 3 cm across, occurring in a mouse six months after injection of the carcinogen. This mouse belonged to group 1, fed a low caloric diet. Photomicrograph, ocular 10, objective 10, Zeiss.

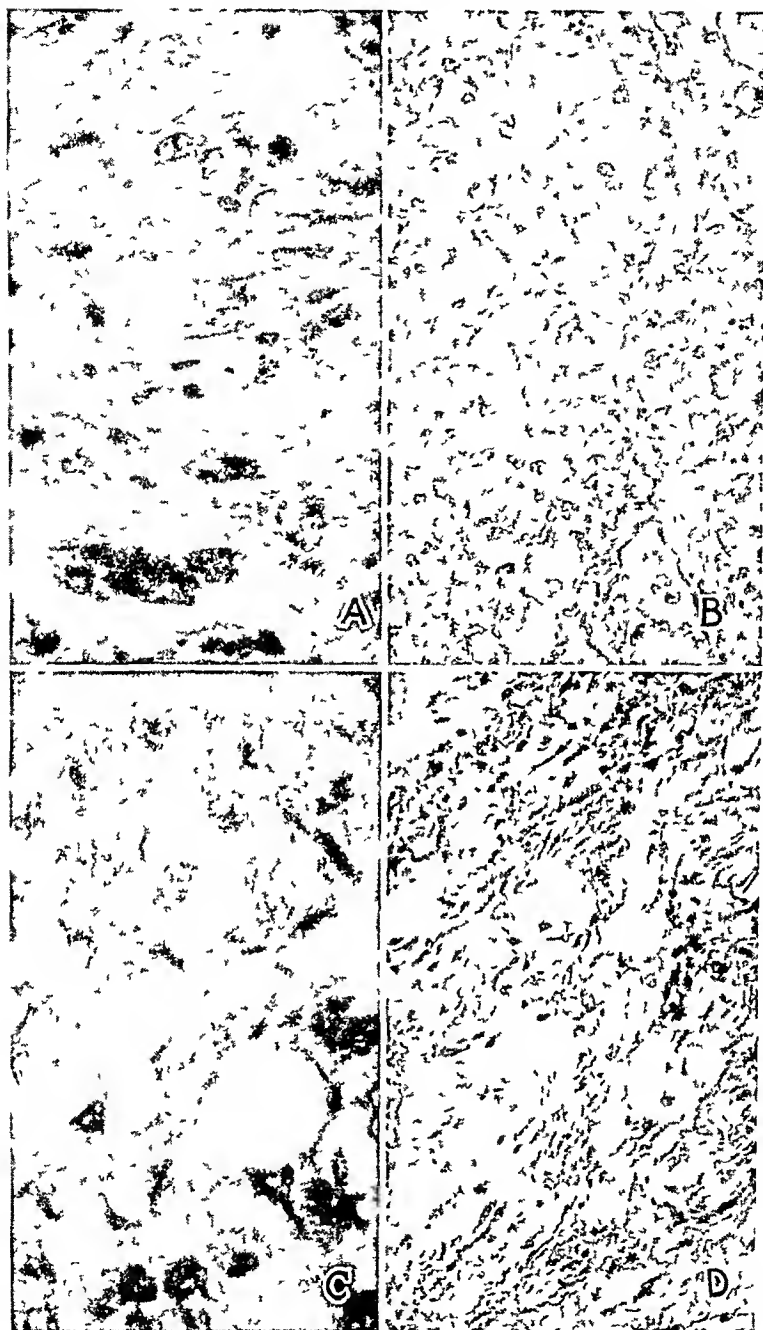


Fig 3—*A*, giant cells containing a single nucleus with a varied number of lobes connected by narrow bands of nuclear substance. In their intimate cytologic characteristics these large cells resemble the small immature granular fat cells, transitional patterns could often be recognized between the two cell types. The tissue was taken from the same tumor as that shown in figure 2*E*. Photomicrograph, ocular 10, oil immersion, Zeiss.

*B*, low power view of a full grown tumor from the interscapular region showing predominance of lipid cells. The tissue was removed from a mouse of group 2, fed a high caloric diet, five months after injection of the carcinogen. Photomicrograph, ocular 10, objective 10, Zeiss.

*(Legend continued on opposite page)*

nucleus, displaced to the periphery, contained several nucleoli and fine purplish violet chromatin granules in a background of pinkish karyoplasm (parachromatin). Against the identifying of this cell with the typical plasma cell of the connective tissue was, however, the less sharp cellular outline, the absence of a clear perinuclear area and the less pronounced cytoplasmic basophilia.

These cells were not conspicuous in the full grown tumor mass, as they appeared to be outnumbered by the other cellular elements, but were instead a predominant feature during the early developmental stages of the growth, they could be detected in the animals killed at the first appearance of the tumor. Distinct transitional patterns noted between these cells and cells with characteristics of histiocytes, on one side, and cells with features of lipoblasts, on the other, led to the impression that they represented an intermediate stage in the development of the lipid cells. The first step in their differentiating along the line of the lipid-storing cells was found to occur in an inversion of the nucleocytoplasmic ratio, to the advantage of the cytoplasm, in a tendency of the nucleus to become centralized, and in an increase in the size of the cell. The lipid storage function of these cells was already apparent at this stage of development, as shown by the presence of small cytoplasmic sudanophilic droplets. A spongy appearance of the cytoplasm, its becoming amphophilic or eosinophilic, and a greater amount of fat droplets appeared to characterize the following stage of development, and when a number of these cells lay close together, they became polyhedral in shape, because of reciprocal compression, and showed a tendency to arrange themselves in clusters. The active part taken by them in the growth was shown by the frequency with which cells with two nuclei could be recognized among the proliferating cells.

*Incidence of New Growth Under Low and Under High Caloric Intake*—The incidence of the growth in the two groups of animals, those fed a low caloric diet (group 1) and those fed a diet of high caloric value (group 2), is shown in table 1. Cancer developed in 7 of the 14 animals of group 1, a slightly higher incidence of growth—10 of 14—was shown by the animals of group 2, but there is less difference between the two groups of animals when it is considered that 3 animals

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*C*, high power view of tissue from the same tumor as that represented in *B*. That the newly formed cells closely resemble the steatoblast or embryonal fat cell of the rodent is apparent. Photomicrograph, ocular 10, objective 40, Zeiss.

*D*, section from another field of the same tumor from which those reproduced in *B* and *C* were obtained, showing a loose-textured framework of myxomatous connective tissue with stellate cells irregularly scattered. Photomicrograph, ocular 7, objective 10, Zeiss.



of group 1 died at the beginning of the experiment in an extremely emaciated condition

The development of the growth at one site of inoculation of the carcinogen did not prevent its development at another site of injection of the same agent, this led to a total of fifteen tumors in group 1 and seventeen in group 2. Growths in symmetric regions—axillas, groins—occurred in 2 animals, once in each group

TABLE 1—*Comparison of Results Obtained with a Carcinogen in a Group of Mice Fed a Low and a Group Fed a High Caloric Diet*

| Animal | Interscapular Region | Site of Injection                        |   | R | Axilla | L |
|--------|----------------------|--|---|---|--------|---|
|        |                      | Groin                                    |   |   |        |   |
|        | Treatment            | Carcinogen in Corn Oil—Low Caloric Diet  |   |   |        |   |
| 1      | —                    | —  | — | — | —      | — |
| 2      | +                    | —  | + | + | —      | — |
| 3      | +                    | —  | — | — | —      | — |
| 4      |                      | Died early in the experiment             |   |   |        |   |
| 5      | +                    | —  | + | + | —      | — |
| 6      | —                    | —  | — | — | —      | — |
| 7      |                      | Died early in the experiment             |   |   |        |   |
| 8      | —                    | +  | + | + | —      | — |
| 9      | —                    | —  | — | — | —      | — |
| 10     |                      | Died early in the experiment             |   |   |        |   |
| 11     | —                    | —  | — | + | —      | — |
| 12     | —                    | —  | — | — | —      | — |
| 13     | —                    | +  | — | — | +      | + |
| 14     | —                    | —  | + | + | —      | — |
|        | Treatment            | Carcinogen in Corn Oil—High Caloric Diet |   |   |        |   |
| 15     | +                    | —  | — | + | —      | — |
| 16     | —                    | +  | — | + | —      | — |
| 17     | —                    | —  | — | — | —      | — |
| 18     | —                    | —  | — | — | —      | — |
| 19     | +                    | —  | + | — | +      | + |
| 20     | —                    | —  | — | — | —      | — |
| 21     | —                    | —  | + | — | —      | — |
| 22     | —                    | —  | — | + | —      | — |
| 23     | —                    | +  | — | + | —      | — |
| 24     | +                    | —  | — | + | +      | + |
| 25     | —                    | —  | + | — | —      | — |
| 26     | —                    | —  | — | — | —      | — |
| 27     | —                    | —  | + | — | —      | — |
| 28     | —                    | —  | + | — | —      | — |

Note + indicates growth — indicates no growth

No significant difference of the two groups of animals was noticed in regard to the time of first appearance of the tumors. Growth was first noticed in the right groin of an animal of group 1 ninety days after the injection of the carcinogen, and six days later a growth was apparent in the left groin of an animal of group 2. From the ninetieth to the one hundred and eighteenth day the growths started to appear in the other animals. Once the tumor had started in any animal it enlarged rapidly

so as to reach a diameter of as much as 3 cm in the course of four to six weeks, no differences were noticed in the rate of growth of the tumor in the two groups of animals

From the foregoing observations the conclusion can be drawn that differences of caloric intake and the ensuing depletion or increase of fat in the depots as realized by the procedures used in the present experiment did not seem to influence in any apparent way the anaplastic growth which resulted from carcinogenic stimulation

TABLE 2—*Comparison of Results Obtained with a Carcinogen Dissolved in Pork Fat and in Corn Oil*

| Animal | Site of Injection                    |                                      |       |   |   |        |
|--------|--------------------------------------|--------------------------------------|-------|---|---|--------|
|        | Treatment<br>Interscapular<br>Region | Carcinogen in Pork Fat—Standard Diet |       |   |   | Axilla |
|        |                                      | R                                    | Groin | L | R |        |
| 29     | —                                    | —                                    |       | — | — | —      |
| 30     | —                                    | +                                    |       | — | — | +      |
| 31     | —                                    | —                                    |       | — | — | —      |
| 32     | —                                    | —                                    |       | — | — | —      |
| 33     | +                                    | —                                    |       | — | — | —      |
| 34     | —                                    | —                                    |       | — | — | —      |
| 35     | —                                    | +                                    |       | — | + | —      |
| 36     | —                                    | —                                    |       | — | — | —      |
| 37     | —                                    | —                                    |       | — | + | —      |
| 38     | —                                    | —                                    |       | — | — | —      |
|        | Treatment                            | Carcinogen in Corn Oil—Standard Diet |       |   |   |        |
| 39     | +                                    | —                                    |       | — | + | —      |
| 40     | —                                    | —                                    |       | + | — | —      |
| 41     | —                                    | +                                    |       | — | — | —      |
| 42     | +                                    | —                                    |       | + | — | —      |
| 43     | —                                    | —                                    |       | — | — | —      |
| 44     | +                                    | +                                    |       | — | + | —      |
| 45     | —                                    | —                                    |       | — | — | +      |
| 46     | +                                    | —                                    |       | + | + | —      |
| 47     | —                                    | —                                    |       | — | — | +      |
| 48     | —                                    | —                                    |       | — | — | —      |

+ indicates growth — indicates no growth

*The Fatty Solvent Carrying the Carcinogen as a Factor in the Incidence of the Growth*—A comparison of the results observed in the animals receiving the carcinogen in corn oil solution and those receiving the same amount of the carcinogen dissolved in emulsified pork fat—both fed the standard well balanced diet—shows some difference in the incidence of the tumor growth (table 2) Carcinoma developed in 4 of the animals receiving the carcinogen dissolved in animal fat and in 8 of those receiving the carcinogen in corn oil solution, with a total of six tumor growths in the former and fourteen in the latter Further indication that the vegetable oil solvent might favor prompter action of the carcinogen was found in the earlier appearance of the growth both

in this group of animals and in the similarly treated animals in which development of the growth was studied under low and high caloric diets. Whereas in the animals receiving the carcinogen dissolved in corn oil of the present series the tumor growth made its first appearance between the ninety-fifth and one hundred and twentieth day, in the animals receiving the same amount of carcinogen dissolved in pork fat the first evidence of incipient growth was noticed at the one hundred and twenty-ninth day after inoculation, and within the successive thirty-seven days the tumor made its appearance in only 3 additional animals of the group. Once the development of the tumor had started, no differences were noticed in the rate of growth between the two groups of animals, the tumor reaching a size ranging to 3 cm in a period of four to six weeks. The possibility of eliciting multiple tumor growths in the same animal, already shown by the previous experimental series, was confirmed in this group of animals, with a higher incidence in the corn oil group. The gross appearance of the tumor and its intimate cytologic characteristics were the same in the two groups of animals, and successful transplants were obtained from both.

Although the small number of animals used in the experiment limits the conclusion, a point worthy of further investigation seems to emerge from these observations—that the composition and the rate of absorption of the fatty solvent carrying the carcinogen may have some bearing on the incidence and on the time of appearance of the elicited new growth.

#### COMMENT

In the face of the known ability of benzpyrene, dibenzanthracene and other related chemicals to induce cancerous change in many different types of tissues, the production of cancerous growths in the present series by injecting the carcinogen in the midst of fat tissue is not surprising.

It is generally accepted that the fat-forming cells are found in embryonal life in two different forms: (1) widely spaced stellate or spindle-shaped cells, which gradually become rounded as they accumulate lipoid material, immersed in a mucoid intercellular substance, (2) lipid cell groups in glandlike lobules of a moruloid or mulberry appearance in the absence of any mucoid intercellular substance. Both forms are reproduced in the cancerous growths arising from lipid cells, hence Ewing's<sup>8</sup> proposal that these growths be classified as of two types: adult liposarcoma and myxoliposarcoma. The adult type is composed of rounded or polygonal lipoblasts of varying sizes and of adult fat cells without myxomatous or fibroblastic tissue taking any active part in the growth. The

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8 Ewing, J. Arch Surg 31 507, 1935

myxoliposarcoma consists of an admixture of adult fat cells and fat-laden fibrocytes embedded in a fibrosarcomatous tissue. The latter was the structure displayed by the tumor produced in the present series.

Among the tumors obtained by comparable experimental procedures, the closest resemblance is found in the liposarcoma produced by Haagensen and Krehbiel<sup>9</sup> in mice and guinea pigs by repeatedly injecting 1, 2-benzpyrene into the subcutaneous fat tissue. In the growth produced by Haagensen and Krehbiel the striking pattern was that of an atypical proliferation of cells resembling steatoblasts and polymorphonuclear giant cells, which in turn could be compared to the giant multinucleated lipoblasts described by Murray<sup>10</sup> in a transplantable liposarcoma of the guinea pig. The main difference between Haagensen and Krehbiel's tumor and the one presented here consists, however, in the more active part played in the latter by fibroblastic elements, hence it is classified as embryonal sarcoma.

Regardless of the difference of caloric intake and of the vehicle of the carcinogen, the greater incidence of new growths (see tables 1 and 2) obtained in the animals of the present series following a single injection of the carcinogen as compared with the rarity with which new growths of a similar type were induced by Haagensen and Krehbiel employing repeated injections of a closely related carcinogen may find an explanation in the higher concentration of the carcinogen and in its having been introduced more directly into the fat deposits in the present series.

Haagensen and Krehbiel<sup>9</sup> concluded their observations by suggesting that the tumor growth had originated from "adult fat cells as the result of their long continued chemical irritation and stimulation." A similar conclusion is not warranted in the case of the growth under consideration. No mitotic figures or signs of direct cell division, and no other patterns suggesting proliferation of adult fat cells were noticed in any of the growths of the present series.

Whether or not the adipose cells are capable of multiplying once they have reached their full maturity is still a point of controversy. This possibility was admitted by Kolliker<sup>11</sup> but was denied more recently by Wassermann,<sup>12</sup> who advanced the opinion that fat cell proliferation occurs through a dedifferentiation by which the adult fat cell is returned to the original pluripotent embryonal reticulum cell capable of giving rise not only to new fat cells but to a variety of connective tissue cells, hematic cells included.

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9 Haagensen, C. D., and Krehbiel, O. F. *Am. J. Cancer* **27**: 474, 1936.

10 Murray, J. A. *J. Path. & Bact.* **20**: 260, 1916.

11 Kolliker, A. *Anat. Anz.* **1**: 206, 1886.

12 Wassermann, F. *Ztschr. f. Zellforsch. u. mikr. Anat.* **3**: 235, 1926.

Without ruling out the possibility that proliferation of fat cells may occur under certain conditions through a subdivision of the nuclei of adult fat cells, as indicated by Kolliker, or through retrogression of the adult fat cells, as thought by Wassermann, personal observations on atrophic fat, on fat tissue under inflammatory stimulation and on spontaneous cancerous fat tissue new growths have led to concurrence in the impression that fat cell proliferation, both cancerous and noncancerous, takes place according to the same plan as that by which in embryonal development undifferentiated mesenchymal cells are brought to mature into fat cells. This is accomplished by the revival of the dormant histiocytic cell of the fat tissue, which under the influence of a variety of stimuli rapidly differentiates into a fat cell, going through the same cellular metamorphosis that the mature fat cell experienced in its development.

Evidence supporting this conception was found also in the early developmental stages of the tumor under consideration, which regardless of the localization seemed invariably to start from a multicentric proliferation of the undifferentiated mesenchymal cells embedded in the fat tissue lobule. Cells lymphocytoid in appearance and larger cells which in many respects resembled the so-called reticular plasma cell of the bone marrow prevailed in these early stages. In this connection it is worth recollecting that cells similar to those under consideration are mentioned in a number of descriptions of the development of embryonal fat tissue. Basing his conclusions on the finding of cells resembling plasma cells in embryonal fat tissue, Waldeyer<sup>13</sup> expressed the view that the wandering plasma cell of the connective tissue can be mobilized for the function of storing fat. Bodritzky<sup>14</sup> advanced a similar opinion, and in the first unitarian conception bearing on the origin of fat cells, Poljakoff<sup>15</sup> concluded that Waldeyer's "plasma cell," Ranvier's "fat cell," Ehrmann's "mulberry cell" and the "fat cell" of the subcutaneous tissue are merely modifications of an embryonal connective tissue cell. More recently Geschickter<sup>5</sup> in reviewing a large series of lipoid tumors stressed the presence in cancerous growths of a cell type resembling a "plasma cell or fetal cartilage" and pointed out the possibility that it might represent the forerunner of the larger foam cell.

The intimate admixture, in the present growth, of cells resembling fibroblasts and of lipoid cells in various stages of development again raises the question, debated so many times, of the genetic relationship of the two cell types.

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13 Waldeyer Arch f mikr Anat 11 176, 1875

14 Bodritzky Centralbl f d med Wissensch 23 753, 1885

15 Poljakoff Arch f mikr Anat 32 122, 1888

Commenting on the origin of the fat cells in a case of lipomyxosarcoma, Jacobson<sup>16</sup> interpreted the clustering of mucin about fat droplets in the cells of the growth as evidence of the close relationship of the fat cell and the fibroblast

Against the assumption that imbibition of fat is a potential function common to all connective tissue cells, fibroblasts included, is, however, the fact of common knowledge that in some parts of the body fat tissue new growths are extremely uncommon and that no matter how great the general adiposity can be, fat does not become deposited in the connective tissue. If the conception that the cell of the fibrous connective tissue may store fat is to be accepted, one has therefore to conceive the existence of two kinds of fibroblasts, one linked to the lipid metabolism and the other independent from it. Along this line is the conclusion of Maximow<sup>17</sup> that the specialized fat cell is entirely distinct from the fibroblast and that both in embryonal and in adult life fat tissue cells are formed from undifferentiated mesenchymal cells situated about blood vessels.

The description by Murray and Stout<sup>18</sup> of a liposarcoma growing "in vitro" includes cellular elements with the cytoplasmic but not with the nuclear characteristics of a fibroblast. Burkhardt<sup>19</sup> also, in cultures of bone marrow of adult guinea pigs, has shown that the capacity developed by the adipose tissue for fat storage is not coincident with the loss of other mesenchymal potentialities, while containing large vacuoles of fat, these cells were still capable of locomotion and phagocytosis, and even of undergoing cell subdivision, at times they appeared as ameboid, wandering cells, at other times as fibroblast-like cells, and occasionally they displayed the appearance of multinucleated giant cells.

The gamut of cells described by Burkhardt<sup>19</sup> and by Murray and Stout<sup>18</sup> is run by the growth described here. The great variability of cellular forms which it exhibited might be regarded as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule, which under carcinogenic stimulation differentiated into a variety of connective tissue cells, of which the lipoblasts were merely an offshoot with distinct characteristics.

#### SUMMARY

Another tumor type is added to the several forms of sarcoma which have been induced by means of carcinogenic hydrocarbons 1, 2, 5, 6-di-

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16 Jacobson, V. C. *J. Cancer Research* **6** 109, 1921.

17 Maximow, B. *Textbook of Histology*, Philadelphia, W. B. Saunders Company, Philadelphia, 1934.

18 Murray, M. R., and Stout, A. P. *Am. J. Path.* **19** 751, 1943.

19 Burkhardt, L. *Arch. f. exper. Zellforsch.* **16** 187, 1934.

benzanthracene injected in the midst of fat tissue deposits of white mice was productive in a large number of animals of a transplantable new growth provided with the characteristics of an embryonal cell liposarcoma. The great variability of cellular forms which it exhibited is regarded as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule which under carcinogenic stimulation differentiated into a variety of connective tissue cells, of which the lipoblasts were merely an offshoot with distinct characteristics. From observations on early new growths evidence was obtained that lipid cell cancerous proliferation takes place according to the same gamut of cellular changes by which in embryonal development undifferentiated mesenchymal cells are brought to mature into fat cells.

Differences in caloric intake and the ensuing depletion or increase of fat in the depots did not seem to influence in any apparent way the incidence and progress of the growth.

The development of a growth at one site of inoculation of the carcinogen did not prevent the development of another growth at another site of injection of the same agent. This resulted in a number of instances in a picture grossly resembling a multicentric lipoblastosis.

## SACCULATED MECKEL'S DIVERTICULUM INCORPORATED IN THE WALL OF THE ASCENDING COLON

M D BOSSE, M D  
PITTSBURGH

**M**ECKEL'S diverticulum, estimated to occur in approximately 2 per cent of persons, is recognized to vary greatly in size, shape, position, attachments and structure

In the case to be described, a peculiar, and perhaps unique, structure and appearance of Meckel's diverticulum prevented recognition of the diverticulum at operation

A white youth 17 years old was admitted to the hospital with the typical symptoms and signs of partial or complete intestinal obstruction of four days' duration. He was severely dehydrated. After an attempt to restore normal water balance, an incision was made through the right rectus abdominis muscle and the peritoneum, with the patient under ether anesthesia. A distended and congested small bowel was encountered. The terminal part of the ileum was twisted and tightly wrapped around what appeared to be an anomalous portion of bowel 4 inches (10 cm) long and  $1\frac{1}{2}$  inch (about 1 cm) in diameter, producing complete obstruction. The anomalous bowel had no mesentery and extended from a point on the terminal part of the ileum about 75 cm above the cecum to another point on the ascending colon just above where the ileum enters into the cecum. It appeared to be a communication between the ileum and the ascending colon. The twisted ileum was loosened, and the anomalous portion of intestine was resected from the ileum and the cecum by electrocautery. The openings were inverted by purse string sutures. The twisted small bowel was dusky in color but appeared viable after release of the obstruction. The abdominal incision was closed tightly in layers. The patient passed gas and some feces by rectum after the operation, and at first his condition seemed improved. However, his abdomen remained distended, and a moderate fever was present. He died six days after the operation, apparently from generalized peritonitis.

An autopsy was performed one and one-half hours after death. In the lower part of the abdomen there was a healing recent incision of the right rectus abdominis, 15 cm long. The peritoneal cavity contained 2,000 cc of cloudy, yellowish gray, foul-smelling fluid, and all peritoneal surfaces were covered by thick, yellow, fibrinous exudate. The small intestine was considerably distended with fluid and gas throughout. The site of resection on the ileum had ruptured and presented an opening 0.5 cm in diameter. This was 72 cm above the ileocecal junction. The cecum, the appendix and the ileum had their normal relationships. On first inspection, the ascending colon exhibited its usual external appearance.

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From the Institute of Pathology and the Landon Surgical Clinic of the Western Pennsylvania Hospital



except for the peritoneal exudate and the presence of a 2 mm-sized opening on the serosal surface, just above the ileum, where the anomalous portion of intestine had been resected. On dissecting this area, however, it was found that the opening did not enter the ascending colon but instead entered a blind pouch 4 cm long, 4 cm wide and 3 cm thick, intimately incorporated in the wall of the ascending colon and covered by peritoneum (fig 1). The pouch contained thick,



Fig 1—Photograph showing the cecum and the ileum with a probe representing the excised portion of the diverticulum. The lateral aspect of the cecum is at the top of the picture. The pouch of the diverticulum has been opened to the right of the probe.

yellow, turbid fluid and the lining resembled intestinal mucosa. The lumen of the pouch was separated from the lumen of the ascending colon by a septum 1.5 cm thick. The floor of the pouch was 1 cm above the ileocecal valve (fig 2).

A schematic representation is seen in figure 3.

Microscopic sections of the pouch showed a mucosal lining suggestive of ileum, which was in great part necrotic, extensively ulcerated and covered with fibrinous exudate. Indications of peritoneal remnants were seen in elastic laminae present in the connective tissue between the muscularis of the pouch and that of

the ascending colon, on the one hand, and the muscularis of the ileum, where the latter entered the cecum, on the other

No other abnormalities were found at the autopsy

The elastic laminae identified between the muscularis of the pouch and surrounding structures indicate that the distal end of this Meckel's diverticulum had been completely invested by peritoneum but had be-

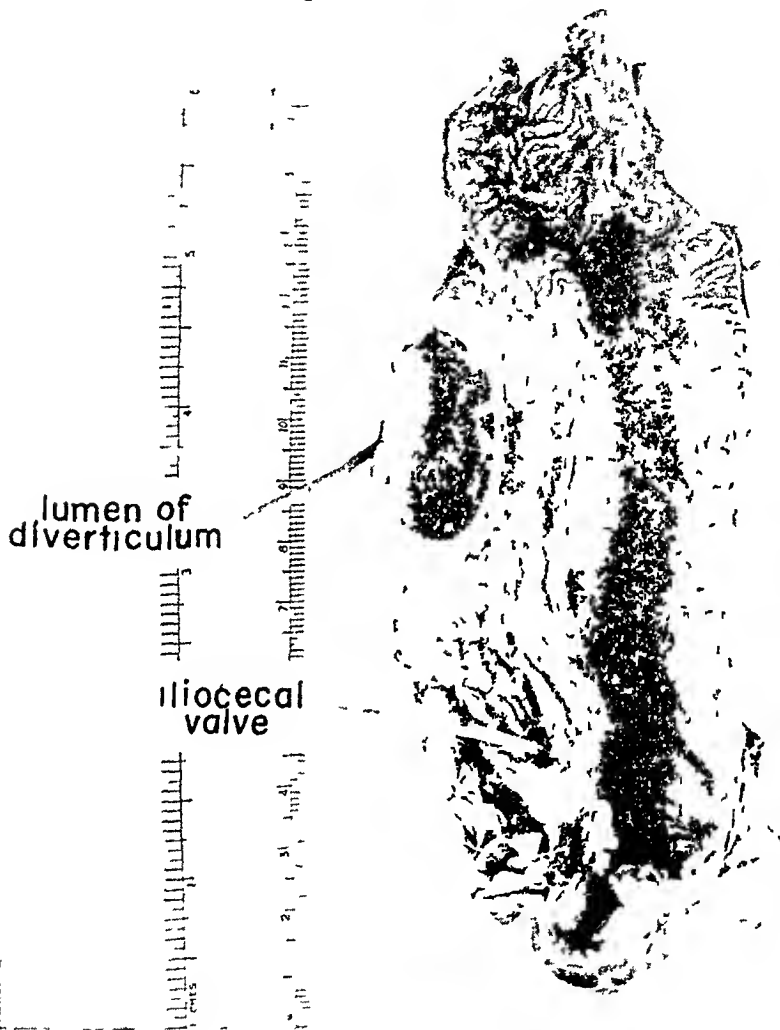


Fig 2—Photograph showing a cross section of the cecum and the ascending colon from a posterior point of view. The medial side of the specimen is to the left and the cecum is at the bottom.

come adherent to the wall of the ascending colon. It either was originally dilated at the distal end or else the dilation occurred after it became incorporated in the wall of the ascending colon. The latter mechanism seems more likely, since partial obstruction may have been caused by the sharp angle at which the proximal part of the diverticulum joined it.

The distal end of the diverticulum was so well incorporated in the wall of the ascending colon that the exact nature of the anomaly was not recognized at operation. It was thought to be an anomalous portion of bowel connecting the lumen of the ileum with that of the ascending colon—a kind of “double terminal ileum.” In fact, even after it was removed at autopsy, its true nature was determined only on subsequent dissection of the specimen.

A review of the literature discloses only 1 case<sup>1</sup> in which a somewhat similar complication of Meckel's diverticulum was described, although

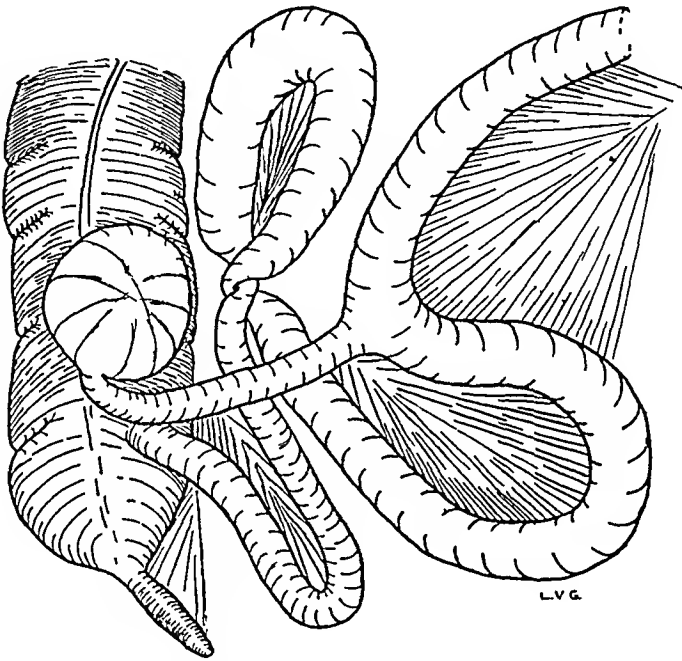


Fig 3—Reconstruction drawing of the condition encountered at operation, with the medial aspect of the cecum and the ascending colon turned anteriorly to expose the diverticulum. The dilated terminal end of the diverticulum is drawn in prominently, although it was not discovered at operation.

the manner in which the diverticulum adhered to the cecum was evidently not that of being intimately incorporated in the wall of the ascending colon as in the present case. Also no mention was made of sacculation of the distal end of the diverticulum. This patient recovered after reduction of the volvulus and removal of the diverticulum and the appendix.

Complications incident to Meckel's diverticula are relatively rare in view of the known frequency of these diverticula. Intestinal obstruction is the most common cause of such complications. In many of the cases it has been due to the presence of a fibrous cord extending from the tip

1 Miller, R. H., and Wallace, R. H. *Ann Surg* 98 713, 1933

of the diverticulum to some other part of the abdomen, usually the umbilicus or the mesentery and abdominal wall, less frequently to other structures

#### SUMMARY

A case in which the saccular dilated distal end of Meckel's diverticulum was incorporated in the wall of the ascending colon is reported. This anomaly was observed in a 17 year old white youth who died of generalized peritonitis following resection of the proximal, unincorporated part of the diverticulum. A volvulus of the terminal part of the ileum was associated with the anomaly. The colonic wall's incorporation of the sacculated distal end of the diverticulum was so complete that this complication was not recognized at operation or on first inspection at autopsy.

## HEMANGIOSARCOMA OF THE SPLEEN

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PRIMARY splenic neoplasms, because of their variety and rarity, are pathologic curiosities. In 1945 Bostwick<sup>1</sup> provided a workable classification, based on cell types, and brought the total of reported splenic tumors of all varieties to 161.

A review of the literature with reference to the neoplasm reported here revealed that there is a small group of primary splenic tumors which are characterized by well defined vascular channels, by areas of sarcomatous aspect histologically, and usually by metastases. This group was recognized by Wright<sup>2</sup> but since these tumors appear under several headings, it seems worth while to reassemble them and to report an additional case.

### REPORT OF A CASE

C. K., a 78 year old white man, was admitted to St. Luke's Hospital for the fourth time June 15, 1945.

His past history revealed yellow fever in 1874, malaria in 1892, a venereal wart in 1900, a "generalized eruption" in 1901 and typhoid fever in 1905. He was admitted in 1935 for a direct inguinal hernia and prostatic hyperplasia. Examination revealed an enlarged prostate gland, an inguinal hernia and a hydrocele. Dilated abdominal veins were noted. A hemogram and the urine were within normal limits. Serologic tests for syphilis revealed none. A roentgenogram of the chest and an electrocardiogram disclosed no abnormalities. Transurethral prostatic resection and bilateral vasotomy were performed. The patient was readmitted in January 1936 for repair of the inguinal hernia, this was followed by uneventful convalescence. In December 1940 he was admitted subsequent to irrigation of the right antrum. At that time crepitus was noted in the infraorbital tissues. This resolved in twelve hours, and the patient was discharged without further study.

From 1940 to 1945 he complained of intermittent pain occurring in the lower part of the back and in the legs. It was associated with increasing fleeting dull pains in all bones and with severe pain in the left upper quadrant of the abdomen. It was described as a dull ache which was occasionally punctuated by sharper pain radiating from the back around both sides of the abdomen above the umbilicus. Weakness and fatigability were prominent symptoms for one month prior to admission.

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From the Department of Pathology, St. Luke's Hospital

1 Bostick, W. L. *Am J Path* 21: 1143, 1945

2 Wright, A. W. *Am J Path* 4: 507, 1928

June 15, 1945, he underwent a sudden syncope and was admitted for observation. Examination revealed a malnourished, pale white man with an icteroid tinge of the skin. The tongue showed no papillary atrophy. There were decreased breath sounds at the apex of the right lung. Marked tenderness of the left costovertebral angle and the left upper quadrant of the abdomen (spleen) was found. The spleen could not be palpated. Moderate prostatic hyperplasia was noted, and also a few small internal and external hemorrhoids. No neurologic changes were observed.

Examination of the urine was noncontributory. The hemogram at admission was as follows: hemoglobin, 5.6 Gm per hundred cubic centimeters, red blood cells, 1,900,000, and white blood cells, 9,700, per cubic millimeter, polymorphonuclears, 86 per cent, lymphocytes, 12 per cent, monocytes, 2 per cent, platelets, 70,000 per cubic millimeter, reticulocytes 0.1 of 1 per cent, hematocrit, 22, color index, 1, volume index, 1.2, mean corpuscular hemoglobin, 29.9 micromicrograms, mean corpuscular volume, 110 cubic microns. Considerable basophilic stippling was noted in smears, marked anisocytosis and poikilocytosis and rare nucleated red cells were observed while the differential count was being made. Wassermann and Kahn tests for syphilis were negative. Chemical examination of the blood showed urea nitrogen, 19.4 mg per hundred cubic centimeters, icterus index, 8, acid phosphatase, 1.8 Gutman units, erythrocyte sedimentation rate, 13 mm per hour (Westergren). The stool showed no blood. Gastric analysis revealed no free hydrochloric acid after histaminic stimulation. Roentgen examination of the chest revealed a mass of homogeneous soft tissue filling the apex of the right lung and extending down to the third rib posteriorly. This appeared to produce destruction of the posterior aspect of the second rib. Pleural exudate filled the costophrenic sulcus on both sides. Barium sulfate studies of the colon revealed no abnormalities.

Admission diagnoses of heat stroke, prostatic hyperplasia and possible pernicious anemia were made. Following roentgen examination of the chest, a diagnosis of generalized carcinomatosis of undetermined origin was entertained.

The patient was given 15 units of concentrated liver on June 21 and on three successive days thereafter. A reticulocyte response of 20.1 per cent was noted on the fifth day of liver therapy. The temperature remained normal, and the patient appeared to be doing well until the thirteenth hospital day, when he died suddenly without obvious clinical reason.

Autopsy twenty-four hours after death showed a well developed but thin 78 year old white man. Seven hundred cubic centimeters of serous fluid was found in the right pleural cavity and 400 cc of similar fluid in the left. The lungs were increased in weight and showed marked hypostatic congestion. There was a small fibrotic area at the right apex. The heart weighed 575 Gm. The myocardium was pale and flabby. The papillary muscles were calcified. There was atherosclerosis of the large vessels. In the abdominal cavity, in the left upper quadrant, was a large mass replacing the spleen and displacing the stomach and the left kidney. The liver (1,700 Gm) showed numerous dark bluish red soft nodules 3 to 5 mm in diameter beneath the capsule, "nutmeg" congestion was prominent and small dark bluish red nodules were scattered throughout all lobes. The gallbladder and the bile ducts were normal. Similar small dark reddish blue nodules were scattered over the parietal and to a lesser extent over the visceral peritoneum. The pancreas, the adrenal glands, the esophagus, the stomach and the duodenum were normal. Meckel's diverticulum was found in the ileum, and the jejunum contained numerous diverticula between the leaves of the mesentery. The appendix was present. The colon was normal except that the splenic flexure was displaced downward by the mass in the upper quadrant.

The spleen weighed 1,700 Gm and measured 19 by 15 by 12 cm. It was adherent to the diaphragm and to the abdominal wall. The capsule was covered by soft fibrous adhesions. The spleen was moderately firm and dark red. The sectioned surfaces showed deep red lobulated areas surrounding areas of hemorrhage and infarction. No normal-appearing splenic tissue remained. The splenic artery and vein and the portal vein were normal. Numerous small, deep red retroperitoneal lymph nodes were noted.

The right kidney was a hydronephrotic cyst with the ureter stenosed at its junction with the pelvis. The left kidney weighed 175 Gm. The bladder was dis-

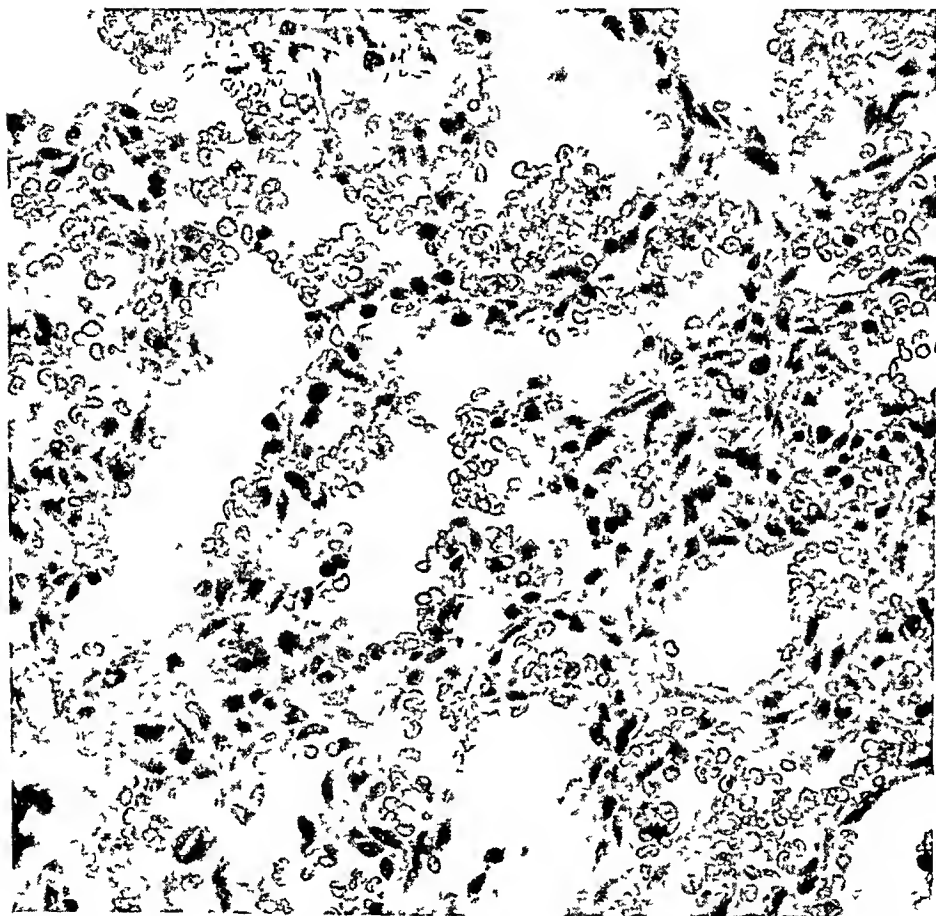


Fig 1—Hemangiosarcoma of the spleen showing elliptic channels and lined with atypical endothelial cells containing blood cells,  $\times 375$

tended with clear urine, and scars were noted near the trigone. There was prostatic hyperplasia of both lateral lobes. All vertebral bodies contained dark hemorrhagic areas with bony rarefaction. The second right rib and the eighth left rib both contained hemorrhagic masses.

Microscopic examination of the lungs revealed an area of fibrosis and scarring with bronchiectases and lymphoid infiltration. The heart showed muscular atrophy. There was chronic passive congestion of the liver. There were numerous small areas of hemorrhage surrounded by a network of small capillary spaces, the majority containing red blood cells. These spaces were lined by spindle-shaped

endothelial cells which appeared larger than the endothelial cells of the hepatic sinusoids and showed considerable nuclear irregularity. These capillaries were thin-walled and closely bound to a loose connective tissue stroma. Surrounding the hemorrhagic area there was considerable polymorphonuclear infiltration, and numerous areas of hepatic necrosis were present. The pancreas and the adrenal glands showed no changes. The spleen disclosed complete loss of normal-appearing reticular tissue in the pulp. No unaltered lymphoid tissue was seen, and the structure was obliterated by the tumor, with areas of hemorrhage, infarction and ex-

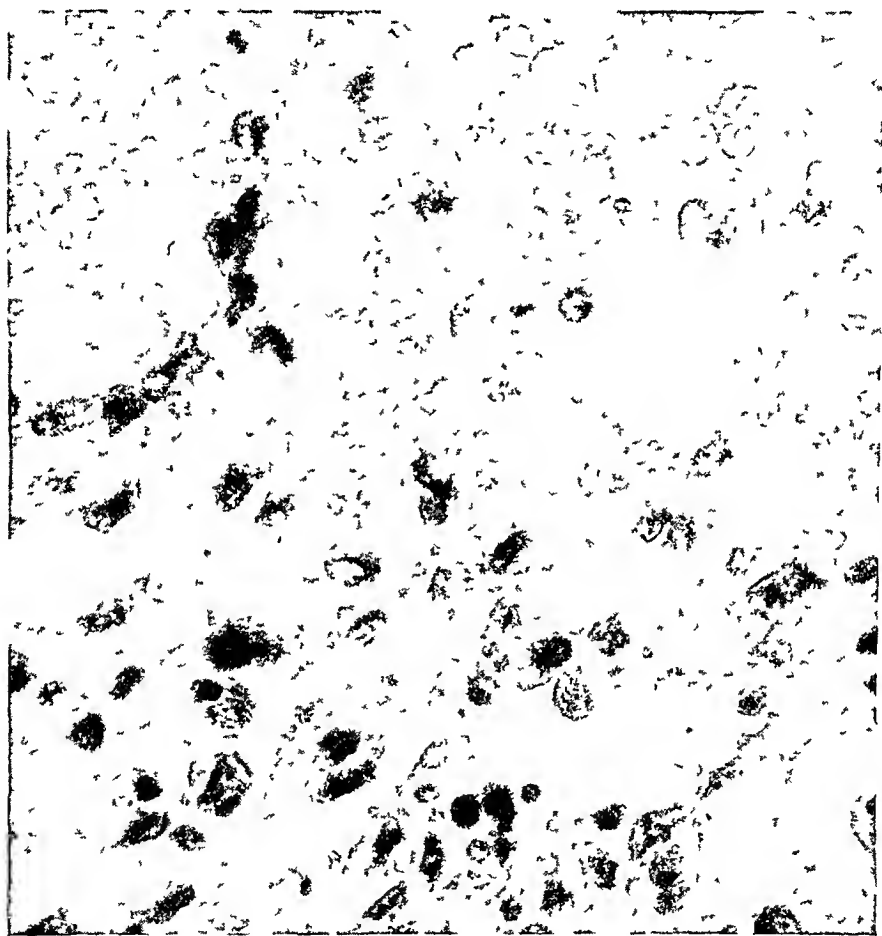


Fig 2—Higher magnification of the neoplasm showing atypical endothelial cells with hyperchromatic nuclei lining vascular channels that contain large numbers of red cells,  $\times 875$

tensive necrosis. In places the tumor was extensively infiltrated by polymorphonuclear leukocytes. Wherever found the neoplasm appeared to have as its dominant characteristic the tendency to form slender elliptic channels and somewhat irregular round cavities, which contained blood cells (fig 1). These channels were lined by plump atypical endothelial cells with hyperchromatic nuclei and rare mitotic figures (fig 2). These cells were in a fine fibrous stroma, which showed considerable variation in different areas. In some portions there appeared little tendency for the vascular elements to become differentiated. Here the cells resembled fibroblasts, and poorly defined vascular channels were observed. Rarely,



projecting papillary processes were found. Sections from parietal and visceral peritoneum showed tumors which were similar in all respects to those seen in the liver and the spleen.

Both ribs and vertebral bodies showed angioma with large areas of transition leading to the area of cells which could not be distinguished from fibrosarcoma (fig 3). Mitoses were infrequent. The marrow was hyperplastic in portions not invaded by tumor.

*Postmortem Diagnosis* — Hemangiosarcoma of the spleen with metastases in the liver, retroperitoneal lymph nodes, the parietal and visceral pleura, ribs and verte-

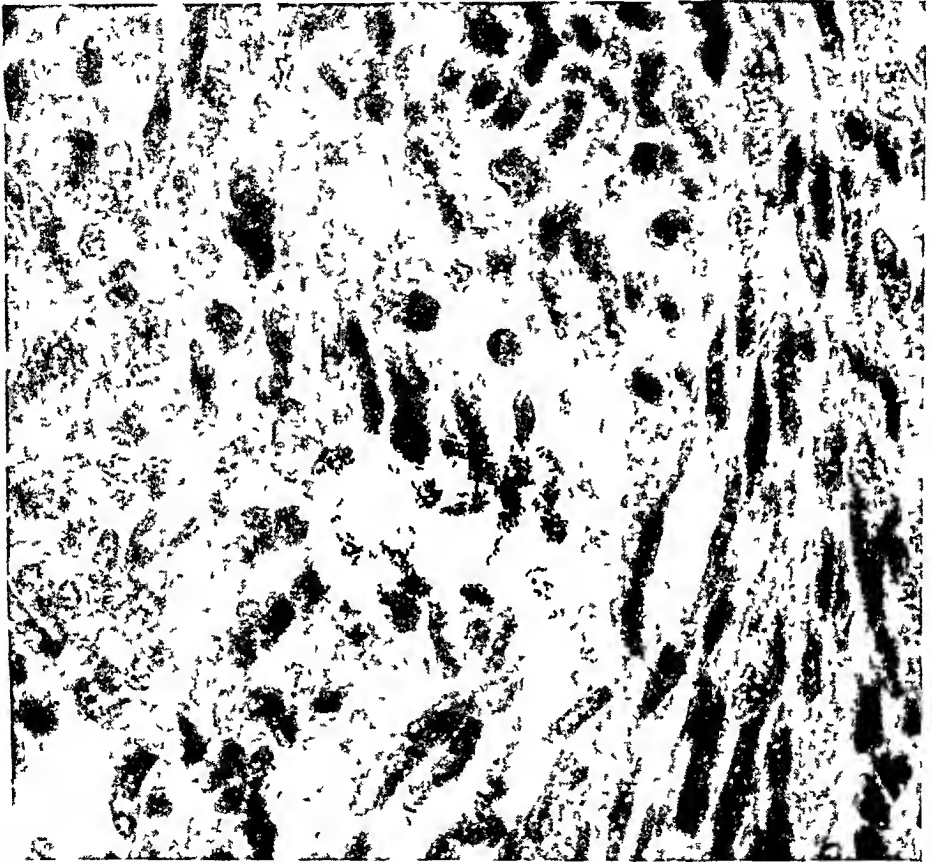


Fig 3 —A tumor area resembling fibrosarcoma,  $\times 875$

bral bodies, prostatic hyperplasia, stenosis of the right ureter and hydronephrosis, arteriosclerosis, diverticula of the jejunum, Meckel's diverticulum, bilateral pleural effusions.

#### PREVIOUSLY REPORTED CASES

CASE 1 (Langhans<sup>3</sup>) —A 30 year old man with a history of trauma three months before death presented a pulsating, rapidly growing tumor in the left upper quadrant of the abdomen, with dyspnea and abdominal pain. The spleen measured 23 by 15 by 10 cm at autopsy. Its surface was nodular, and vessels were observed forming cavernous spaces. Microscopically, hyperplastic endothelium

3 Langhans, T. Virchows Arch f path Anat 75 273, 1879

which was continuous with the sinusoidal endothelium was observed. The cells were described as polyhedral in form. Fibrous and lymphoid tissues did not appear proliferated. A similar growth was found in the liver.

Langhans suggested the possibility of a primary tumor of both liver and spleen but reported it as a primary angiosarcoma of the spleen.

The possibility of a multicentric growth cannot be ruled out. Grossly and histologically it resembled the tumor reported here. Theile<sup>4</sup> and Wright<sup>2</sup> regarded it as similar in all respects to the tumors described by them.

**CASE 2 (Theile<sup>4</sup>)**—Four angiomatous tumors were reported. One was a cancer occurring in a 56 year old man who died of hemorrhage following splenectomy. The spleen weighed 2,500 Gm and measured 25 by 16 by 8 cm. There were adhesions and hemorrhagic areas where angiomatous portions extended to the surface. The splenic tissue was replaced by white areas in the pulp, vascular spaces and numerous blood clots. It was found to consist of a network of cavities containing blood and masses of endothelial cells that appeared like "vessel sprouts." Angiomatous areas showed transition to sarcomatous portions with spindle-shaped or round cells and numerous mitotic figures. Metastases were found in the lung, the stomach and the liver.

Theile's case resembled Wright's and mine in that the tumor formed vessel sprouts and showed areas of rapid growth, anaplasia and numerous mitotic figures. Theile assumed an anaplastic proliferation of angioblasts and was justified in calling this tumor sarcomatous angioma. He believed that it was not hemangiosarcoma, since the sheets of spindle cells contained no red blood cells.

**CASE 3 (Jores<sup>5</sup>)**—A 45 year old woman died of cardiac failure. Hepatomegaly and splenomegaly had been noted eleven months prior to death. A hemogram showed leukopenia. The spleen weighed 3.6 Kg and measured 31 by 15 by 16 cm. The surface was scarred, and there was obliteration of the splenic structure by a soft, structureless reddish gray mass. A hemorrhagic area was found at the upper pole. Radiation necrosis was prominent. Examination revealed a meshlike tissue with small connective tissue bridges lined with endothelial cells and containing red blood corpuscles. Jores felt that a transition zone was demonstrable from angiomatous to sarcomatous areas. The liver weighed 8.6 Kg and contained numerous dark periportal metastatic nodules resembling the splenic tumor.

Jores was convinced that the splenic tumor was primary. He pointed out that it was the first to appear clinically, and that the lesion of the liver was unlike primary angioma of that organ. Furthermore, it showed a distinct periportal distribution which suggested hematogenous spread from the spleen. Jores expressed the belief that this tumor was similar to those of Langhans and Theile. According to Wright, it was similar to the one he reported. This tumor showed a greater tendency toward angiomatous growth than that of Theile and more distinct metastases than that of Langhans. Dispersed red blood cells and a supporting connective tissue wall for endothelial cells with varying grades of differentiation makes this case resemble the case herein reported.

**CASE 4 (Wright<sup>2</sup>)**—A 25 year old man complained of pain and abdominal swelling of four weeks' duration. There were abdominal distention and epigastric tenderness. There was a leukocyte count of 14,000 with a normal differential count. There was no other alteration from normal in the hemogram. Bile was present in the urine, and clinical jaundice was noted three days after admission.

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4 Theile, F. S. *Virchows Arch f path Anat* 178:296, 1904.

Jores, L. *Zentralbl f allg Path u path Anat* 19:662, 1908.

At laparotomy the spleen and the liver were seen to be greatly enlarged. The patient died postoperatively. The spleen weighed 520 Gm and measured 15 by 5 by 8 cm. Clotted blood and dark reddish nodules were noted on the surface. A pedunculated spherical tumor was found fixed to the spleen. This mass was composed of large cavities filled with dark bloody material. Sections revealed many small nodular hemorrhagic tumors. The liver weighed 4,250 Gm and the capsular surface was studded with raised nodular masses which resembled the splenic tumors. The parenchyma was infiltrated with similar masses. Examination of the splenic tumors revealed a neoplasm characterized by the formation of narrow channels filled with blood. The endothelial lining of these channels was atypical and undifferentiated. Projecting papillary processes were observed in the vascular spaces, and from these typical tumor cells were seen to grow. Numbers of red blood cells were seen within the vascular spaces. There was variation in the degree of anaplasia, mitoses were numerous, and areas of rapid growth were described. The lesions of the liver resembled those of the spleen, and their distribution was periportal.

This tumor resembled the one reported here in its tendency to form vascular spaces. It was, however, a more rapidly growing neoplasm. As pointed out by Wright, it is like the tumor reported by Theile.

CASE 5 (Paine<sup>6</sup>) —The patient was a 64 year old white man with a sixteen weeks' complaint of dyspnea, lassitude and left-sided abdominal pain. A hemogram suggestive of pernicious anemia was reported. The patient died in cardiac failure. At autopsy the liver (2,400 Gm) was congested and was studded with small dark nodules. The spleen weighed 1,380 Gm. The surface was thickened by perisplenitis and adhesions. The surface was dull yellowish red, and areas of hemorrhage were observed. A metastatic lesion was found in the left femur. Microscopic examination showed a splenic tumor composed of cells varying from long spindle-shaped cells with deeply hematophilic nuclei to nearly circular cells with vesicular nuclei, some of which bore close resemblance to endothelial cells. Mitoses were rare. Red blood cells were observed throughout the tumor. Lesions in the liver and the marrow were similar.

Paine felt that the tumor was of endothelial origin because there was evidence of phagocytosis in the tumor cells, because there was a tendency to form tubules and because fibrils were seen developing from the cells.

CASE 6 (Bockelmann<sup>7</sup>) —A 1½ year old child who had nine hemangiomas removed from the skin and the mucous membranes had the spleen removed for tumorous swelling. A large reddish yellow angiomatous tumor was found. Microscopic examination revealed large endothelial cells lining vascular spaces, and some definitely sarcomatous and angiomatous areas. No metastases were found.

In view of the early age of the patient and the possibility that young proliferating mesenchymal tissue may be confused with sarcoma, it is questionable that this case should be included, although it possesses many characteristics exhibited by the present tumor.

The descriptions provided by Albrecht<sup>8</sup> and Fuhrer<sup>9</sup> are incomplete and are not included. The case reported by Snodgrass,<sup>10</sup> which appears to have been

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6 Paine, C. G. *J. Path. & Bact.* 34: 139, 1931.

7 Bockelmann, T. W. A. *Ueber ein Angiom der Milz*, Dissert., Griefswald, H. Adler, 1906.

8 Albrecht. *Ztschr. f. Heilk.* 15: 81, 1856.

9 Fuhrer. *Arch. f. phys. Heilk.* 15: 81, 1856.

10 Snodgrass, T. J. *Surgery* 15: 988, 1944.

one of perendothelioma, and that of Ernst,<sup>11</sup> which was an instance of an eruptive angiomatous tumor, are not included because they resemble only remotely the tumors included in this report

## COMMENT

The question of metastases derived from angioma has received considerable attention in the literature (Willis<sup>12</sup> and Pines and Rabinovitch<sup>13</sup>) Ewing<sup>14</sup> established that in some instances angioma may be multicentric, without metastases entering the picture This is demonstrated in reports of multiple tumors in young persons in whom angioma showed a systemic distribution (Schmitt,<sup>15</sup> Jaffé<sup>16</sup> and Wollstein<sup>17</sup>) or in whom the possibility of a diffuse disease of the reticuloendothelial system was considered (Schlopsnies<sup>18</sup> and Eigler<sup>19</sup>)

In the cases collected here the exclusion of tumors of multicentric origin was not difficult A primary splenic tumor was observed in cases 1, 2, 3, 4 and 5 and in the case reported The dissemination of the tumor to include more than one systemic form of distribution is suggested by the location of the metastases in cases 2, 4, 5, 6 and the case which I have reported The weight of the evidence leads one to presume that in each of these cases the metastases were blood borne from a primary splenic neoplasm

The latitude of terminology in classifications of primary splenic cancers shows that these tumors present particular difficulties from the morphologic point of view and that they do not fit into the pattern of nomenclature accepted for tumors of other organs This may be due to the cytologic variations observed in endothelial cells when these occur in tumors of lymphoid organs (Ewing<sup>14</sup>) It may also be attributed to the infrequency with which these tumors are seen by any one observer

Weichselbaum<sup>20</sup> considered splenic sarcoma as of three types spindle cell sarcoma, endothelial sarcoma and lymphosarcoma In 1923 Smith and Rush<sup>21</sup> broadened this initial classification by dividing all splenic

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11 Ernst, P *Verhandl d deutsch path Gesellsch* 15 232, 1912

12 Willis, R A *Spread of Tumors in the Human Body*, London, J & A Churchill, 1934, pp 148-151

13 Pines, B, and Rabinovitch, F *Arch Path* 33 487, 1942

14 Ewing, J *Neoplastic Diseases*, ed 4, Philadelphia, W B Saunders, Company, 1940

15 Schmitt, L *Centralbl f allg Path* 145 27, 1916

16 Jaffé, R H *Arch Path* 7 44, 1929

17 Wollstein, M *Arch Path* 12 562, 1931

18 Schlopsnies, W *Virchows Arch f path Anat* 85 274, 1881

19 Eigler, G *Ztschr f Kreislaufforsch* 22 249, 1900

20 Weichselbaum, A *Virchows Arch f path Anat* 85 554, 1881

21 Smith, C E, and Rush, G Y *Arch Surg* 7 371, 1923

neoplasms into derivatives of capsule or trabeculae, derivatives of lymphoid elements and derivatives of vascular constituents. As Bostick<sup>1</sup> pointed out, splenic tumors probably should be classified in accordance with the different cell types found in that organ. He listed seven basic neoplastic types.

From Bostick's<sup>1</sup> discussion, however, it is not clear whether he meant to classify all cancerous vascular tumors under the loose heading of angioendothelioma, or whether he would admit cancers in which the unit appeared to be the vessel rather than the cell, angiosarcoma in fact, as a subdivision of this first group. One might question whether such a distinction can or should be made in a group of tumors in which the classic characteristics are ill defined. It is important, as Wright<sup>2</sup> pointed out, to recognize the fact that there exists a group of primary splenic neoplasms which show clearcut differentiation toward well defined vessel formation, i.e., tumors in which the vessels appear to constitute the units of the tumor, and in which many characteristics of sarcomatous tissue are manifested in the morphologic aspects of the tumor cells. This type might well be regarded as separate and distinct from the more commonly described hemangioendothelioma in which the unit appears to be the endothelial cell, vascular spaces are poorly defined, and the tumor cells tend to appear in sheets and masses, characteristic of endothelioma.

In attempting to analyze these tumors from the point of view of their origin and composition, one is confronted with the resemblance they bear both to endothelioma and to sarcoma. Their hemangiomatous properties are evident on gross examination. Microscopically, the tumor is composed of newly formed vessels which encompass fairly large numbers of red blood cells.

Their resemblance to tumors of endothelial origin is manifested by their similarity to Connor's<sup>22</sup> first type of endothelioma in which there is a differentiation toward vascular tissue with sinus formation and the production of vascular spaces. The small cuboidal cells which line the vascular spaces are endothelial cells and the intimate relationship noted between these cells and the supporting stroma is suggestive of the endothelial origin of these tumors.

These tumors cannot be separated from sarcoma in that they reproduce functioning blood vessels. Spindle cells have been described as numerous, as well as many cells which might be considered angioblastic. The nuclei of the sarcoma-like portions of the tumor exhibit in size and form the wide variations characteristic of sarcoma. One might regard this property as consistent with the dual tendency of endothelial cells in lymphoid structures to show epithelial qualities and the potentialities

of connective tissue with reticulum formation penetrating the cell masses and outlining the vessel walls. On the other hand, the connective tissue framework of the developing blood vessels and the absence of sheets of endothelial cells incline to the diagnosis of sarcoma.

The term "angiosarcoma" has been used to designate all tumors originating from blood and lymph vessels and might be applied to these tumors. The subdividing of angiosarcoma into perithelioma (Hildebrandt<sup>24</sup>) and periendothelioma (Borrmann<sup>25</sup>) does not apply to this tumor, since the tumor cells neither encircle nor radiate from the developing vessels.

In my opinion they should be regarded as capillary hemangiosarcoma until such time as the differentiation between endothelioma and sarcoma can be made with certainty and the exact histogenesis of the constituent cells be established.

An incidental finding of importance, and one which has received little attention in the literature, is that alterations occur in the hemograms of patients with splenic cancer (Cases of Howard,<sup>26</sup> Frank,<sup>27</sup> McNee,<sup>28</sup> Shennan<sup>29</sup> and Paine<sup>6</sup> and my own case). In the reported hemograms one notes morphologic changes in the red blood cells which are suggestive of pernicious anemia, i.e., anisocytosis, poikilocytosis, polychromasia and occasional nucleated red blood cells with leukopenia and thrombopenia. This resemblance occurring without the neurologic changes or the glossitis of pernicious anemia and with or without achlorhydria was pointed out by Howard in reporting his case of splenic lymphosarcoma. These changes have been reported frequently enough in cases of primary splenic cancer to be of diagnostic aid in cases of suspected splenic tumor. Both Matas<sup>30</sup> and Grove<sup>31</sup> implied that hemangiomatous growths increase splenic function, whereas, in a case such as mine it would appear that a tumor replacing, invading and compressing normal splenic pulp would tend to decrease rather than to increase splenic function. The exact relationship of the changes of the hemogram

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23 Footnote deleted by the author

24 Hildebrandt, C. L. *Deutsche Ztschr. f. Chir.* **31** 263, 1891

25 Borrmann, C. L. *Virchows Arch. f. path. Anat.* **157** 297, 1899

26 Howard, T. J. *Lab. & Clin. Med.* **14** 1157, 1929

27 Frank, L. *Am. J. M. Sc.* **183** 77, 1932

28 McNee, J. W. *J. Path. & Bact.* **39** 83, 1934

29 Shennan, J. *J. Path. & Bact.* **15** 139, 1914

30 Matas, R., in Piersol, G. M., and Bortz, E. L. *Encyclopedia of Medicine*, Philadelphia, F. A. Davis Company, 1934, vol. 12, p. 843

31 Groves, E. W. H., cited by Campbell, W. C., in Piersol, G. M., and Bortz, E. L. *Encyclopedia of Medicine*, Philadelphia, F. A. Davis Company, 1934, vol. 7, p. 640

and splenic cancer cannot be explained in the present knowledge of splenic function. Furthermore, the changes do not seem to be related to the cell type of the invading tumor, since the reported cases are of several histologic varieties.

#### SUMMARY

The capillary hemangiosarcoma of the spleen presented here is one of a small group of rare primary splenic neoplasms which are characterized by formation of vascular channels, by areas of sarcomatous aspect morphologically and by metastases. It was associated with changes in the hemogram suggestive of pernicious anemia, and terminated the life of the patient within four years after the onset of symptoms which, in retrospect, were suggestive of splenic involvement.

# CONGENITAL RHABDOMYOMATOSIS OF THE HEART

Report of a Case with Autopsy

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MINNEAPOLIS

CASES of the clinical syndrome of congenital idiopathic cardiac hypertrophy can be separated as a rule into four main groups. The first and most common are those in which the hypertrophy is associated with endocardial sclerosis and myocardial fibrosis. A second group includes those rare cases in which the enlargement is due to true hypertrophy of the muscle fibers. Glycogen storage disease of von Gierke accounts for the third group. A remarkable feature is the unusual stability of the glycogen stored in the liver and other tissues, regardless of fixation and time after death. The fourth rarest cause of this syndrome is found in those cases in which the enlargement of the heart is due to localized tumors or a diffuse tumor of the heart composed of pale-staining, vacuolated cells resembling embryonic muscle fibers. A variety of names have been used in recent years to describe these tumors. The earlier authors described them as rhabdomyoma. Von Recklinghausen<sup>1</sup> is generally credited with reporting the first case, in 1862. Some of the early cases were reported by Knox and Schorer<sup>2</sup> in 1906 and Wolbach<sup>3</sup> in 1907. In more recent years cases have been reported by Farber,<sup>4</sup> Yater,<sup>5</sup> Ill and Gray,<sup>6</sup> Wegman and Egbert,<sup>7</sup> Olsen and Cooper,<sup>8</sup> Hillman,<sup>9</sup> Batchelor and Maun<sup>10</sup> and Haymond and Giordano,<sup>11</sup> Batchelor and Maun<sup>10</sup>

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From the University of Minnesota Teaching Service of Northwestern Hospital and the Department of Pathology of the University of Minnesota Medical School, Minneapolis

- 1 von Recklinghausen. *Monatschr. f. Geburtsch.* 20:1, 1862.
- 2 Knox, J. H. M., and Schorer, E. H. *Arch. Pediat.* 23:561, 1906.
- 3 Wolbach, S. B. *J. M. Research* 16:495, 1907.
- 4 Farber, S. *Am. J. Path.* 7:105, 1931.
- 5 Yater, W. M. *Arch. Int. Med.* 48:627, 1931.
- 6 Ill, C. H., and Gray, F. W. *Am. J. Obst. & Gynec.* 28:264, 1934.
- 7 Wegman, M. E., and Egbert, D. S. *J. Pediat.* 6:818, 1935.
- 8 Olsen, R., and Cooper, R. J. *Am. J. Path.* 17:125, 1941.
- 9 Hillman, R. W. *Brooklyn Hosp. J.* 3:181, 1941.
- 10 Batchelor, T. M., and Maun, M. E. *Arch. Path.* 39:67, 1945.
- 11 Haymond, J. L., and Giordano, A. S. *Am. J. Clin. Path.* 16:651, 1946.



made a thorough review of the literature in 1946 and collected 63 cases. They preferred the term "glycogenic tumors of the heart." Most of the recent articles have all stressed the glycogen content of the tumors. However, in the greater majority of the reported cases attempts to demonstrate glycogen in the hearts were unsuccessful. This failure has been attributed largely to lack of alcoholic fixation of the tissues. In 35 or 65 cases collected from the general literature, including our own, the patients were children under 1 year of age. Only in 7 cases were the patients children over 15 years of age.

The case now reported is to my knowledge the first encountered in the department of pathology of the University of Minnesota in over 52,000 autopsies. It is of interest because of the failure to demonstrate glycogen in the tumor cells after alcoholic fixation.

#### REPORT OF CASE

A girl was born at Northwestern Hospital on Sept 10, 1947. She weighed 5 pounds and 10 ounces (2,551.5 Gm) at birth. The delivery was normal. It was the mother's second pregnancy. The first child was living and well. The newborn baby appeared normal during the first few days of life. There was no cyanosis. She failed to gain weight and ate poorly. Her weight on leaving the hospital on the sixth postnatal day was 5 pounds and 10 ounces. She was readmitted October 24, at the age of 6½ weeks, to the service of Drs. Erling Platou and Richard Tudor, because of an infection of the upper respiratory tract. She was poorly nourished. The examination otherwise disclosed no abnormality. She showed some gain in weight and was discharged as improved on October 28. Her appetite remained poor. On November 11 the mother noted that the baby's breathing was irregular and that she could not be aroused. She was brought again to the hospital on November 11 at 4:30 a.m., cyanotic and comatose. Her temperature was 97.8 F rectally, the pulse rate was 140 per minute, and the respirations were irregular and shallow at 15 to 20 per minute. There was intrasternal and suprasternal retraction of the chest. The breath sounds were faint. The resonance of the chest was increased. The cardiac rate and rhythm were regular. Murmurs were not heard. The heart appeared enlarged. The edge of the liver was down 5 cm below the right costal margin. The baby was limp, with poor muscular tone. A roentgenogram of the chest showed diffuse cardiac enlargement, the heart occupying most of the thoracic cavity. The baby was given oxygen, caffeine, sodium benzoate, epinephrine hydrochloride and adrenal cortex extract. Laryngoscopic examination showed the upper respiratory passages to be clear. The baby was given 1 cat unit of lanatosid C (cedilanid<sup>R</sup>) and treated with a penicillin spray, but she remained comatose and died November 11, at the age of 2 months.

*Autopsy* (four and a half hours after death) —The body was that of a poorly nourished, well developed white girl, measuring 53 cm in length and weighing 2,880 Gm. Edema, jaundice and rigor were absent. There was cyanosis of the lips and the finger tips.

The peritoneal cavity was empty, and the surfaces were smooth. The edge of the liver was down 3 cm below the right costal edge and 4.5 cm below the xiphoid process. Each pleural cavity contained about 7 cc of clear straw-colored fluid. The pericardial sac appeared normal. The transverse cardiac diameter was 8 cm,

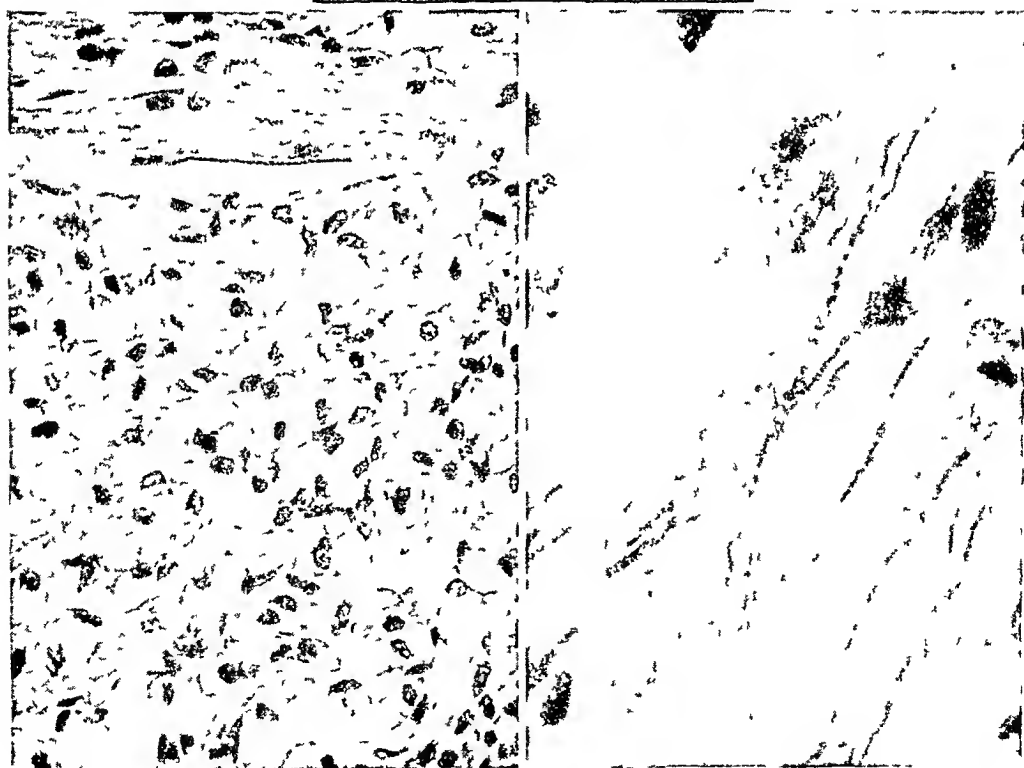


Fig 1—Heart with nodular elevations on the anterior surface of the left ventricle

Fig 2—Photomicrograph in which the pale, granular cells of the tumor may be compared with normal cardiac muscle

Fig 3—Photomicrograph showing cross striations in tumor cells, iron-hematoxylin stain

compared with a transverse thoracic diameter of 9.3 cm. The heart filled the greater part of the thoracic cavity.

The heart weighed 66 Gm. The anterior surface of the left ventricle was covered by several flat, ill defined yellowish brown tumors measuring up to 1.5 cm in size (fig 1). Both ventricles were involved by yellowish brown, irregular tumors, but most of the tumors were confined to the left ventricle. The auricles appeared normal. The appendages and the valves appeared normal. The endocardium, particularly that of the left ventricle, was pale. The arch of the aorta appeared normal. The ductus arteriosus was closed. The coronary orifices and vessels appeared normal.

The lungs were dark red, and crepitation was slightly decreased. The right lung weighed 27 Gm and the left 20 Gm. The spleen weighed 9 Gm and appeared normal. The liver weighed 115 Gm. Its color was a homogeneous yellowish brown. The gallbladder, the gastrointestinal tract, the pancreas and the adrenal glands appeared normal. The kidneys weighed 15 Gm each and showed nothing of note. The bladder and the genital organs presented no anomalies. The thymus weighed 21 Gm. The aorta was smooth. Permission to examine the brain was not obtained.

*Microscopic Examination*—Sections of the heart stained with hematoxylin and eosin showed the myocardium of the septum and of both ventricles to contain circumscribed tumors composed of large, pale, granular cells. The cells were much larger than the normal muscle fibers (fig 2). The cell boundaries were indistinct. The tumor cells contracted strikingly with the normal muscle. Iron-hematoxylin stains showed some of the tumor cells to contain distinct cross striations (fig 3). Best's carmine stain on heart muscle fixed in absolute alcohol at the time of autopsy showed absence of glycogen in the tumor cells. Frozen sections of the heart muscle stained with sudan III showed that the tumor cells contained fat droplets within their cytoplasm.

Best's carmine stain on sections of the liver fixed in absolute alcohol at the time of autopsy showed a normal amount of glycogen in the liver cord cells in the centers of the lobules. The liver appeared normal in sections stained with hematoxylin and eosin. Section of the remaining organs revealed nothing of note.

#### COMMENT

Whether these tumors represent true neoplasms, developmental anomalies, metabolic disturbances or degenerative phenomena has been a point of discussion since the first report of a case was published. Wolbach<sup>3</sup> considered the tumor a true neoplasm as a result of the demonstration of early intracellular fibril formation. Many have stressed the fact that the large, pale, vacuolated cells resemble embryonic muscle. The separation between the various types of hyperplasia and neoplasm is so indefinite that one cannot say whether these tumors should be described as neoplasms. The term "glycogenic tumors of the heart" appears to be a poor one. Batchelor and Maun<sup>10</sup> stated that glycogen was demonstrated in only 8 of the 63 cases collected from the literature. The fact that rhabdomyoma of the heart is associated, in approximately 50 per cent of cases, with tuberous sclerosis seems particularly significant. In many of the other cases, cysts and renal tumors, tumors of sebaceous glands, harelip, cleft

palate, multiple glioma of the brain and other malformations were recorded Hueper<sup>12</sup> considered this an evidence that congenital rhabdomyoma of the heart represents a partial manifestation of a generalized developmental disturbance affecting various organs of the body The tumors of the heart could be best described as a developmental disturbance, or hamartoma In none of the reported cases was evidence of continuous neoplastic growth or ability to metastasize ever shown There seems to be little to support the hypothesis that the tumors represent degenerative phenomena of the heart muscle The term "rhabdomyoma" or "rhabdomyomatosis" is still to be preferred to those linking the tumors to disturbances of glycogen metabolism The tumors bear considerable histologic similarity to myoblastoma occurring in other portions of the body

#### SUMMARY

In a 2 month old infant dying of heart failure, autopsy showed diffuse rhabdomyomatosis of the heart The tumors are interpreted as hamartoma

Touro Infirmary

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12 Hueper, W C Arch Path 19 372, 1935

## BASAL CELL CARCINOMA WITH METASTASES

### A Report of Two Cases

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**B**ASAL cell carcinoma, though frequent, rarely metastasizes, even to nearby lymph nodes. Cases in which metastases are found in distant organs are therefore worthy of note.

Nine such cases have been reported. Korb<sup>1</sup> reported a case in which massive invasion of cervical lymph nodes occurred seventeen months after treatment of a primary basal cell carcinoma of the right temple. Beadles<sup>2</sup> observed, in a 46 year old man, a primary tumor of the face with metastases in submaxillary lymph nodes. Finnerud<sup>3</sup> presented 2 cases. In one a 39 year old man had a tumor of thirteen years' duration on the face, which repeatedly metastasized to cervical lymph nodes, in the other a 56 year old man with primary basal cell carcinoma of the cheek of thirty years' duration showed spreading of the tumor, at that late date, to submaxillary nodes. Eller<sup>4</sup> presented before the Manhattan Dermatological Society a 37 year old man who three years previously had a sore on one finger of the right hand, which was diagnosed as "epithelioma from inner sheath of hair shaft." The finger was amputated, and the histologic diagnosis was "basal cell carcinoma." Twenty-five months later a lump was removed from the right axilla, which the pathologist called "advanced squamous cell carcinoma." A year later, three lesions appeared on the forearm, two of which yielded biopsy specimens which were diagnosed as "typical basal cell carcinoma." On reviewing the axillary lymph node, the pathologist called it "basal cell carcinoma." Forty-five months after the first biopsy,<sup>5</sup> the man was presented again to the aforementioned society, still alive but with a badly swollen arm. Niles<sup>6</sup> reported the death of this patient after amputation of the arm and recurrence of the tumor in the supraclavicular lymph nodes. No autopsy was done.

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1 Korb, H. Arch f klin Chir 97 752, 1912

2 Beadles, C. F. Tr Path Soc London 45 176, 1894

3 Finnerud, C. W. J A M A 82 775 (March 8) 1924

4 Eller, J. J. Arch Dermat & Syph 19 302 (Feb) 1929

5 Eller, J. J. Arch Dermat & Syph 21 900 (May) 1930

6 Niles, H. D. Am J Cancer (supp) 15 2341, 1931

De Navasquez<sup>7</sup> recorded the case of a 44 year old white woman with a "pea-sized" nodule in the skin of the midforehead. On three occasions it was incised, and only after five and one-half years was it removed, having attained a size of 2.5 by 2 cm. At that time it was fixed to both skin and bone. The histologic diagnosis was "basal cell carcinoma." A course of roentgen therapy was given. Eight months later the patient complained of bone pain and was very anemic. She died six years and four months

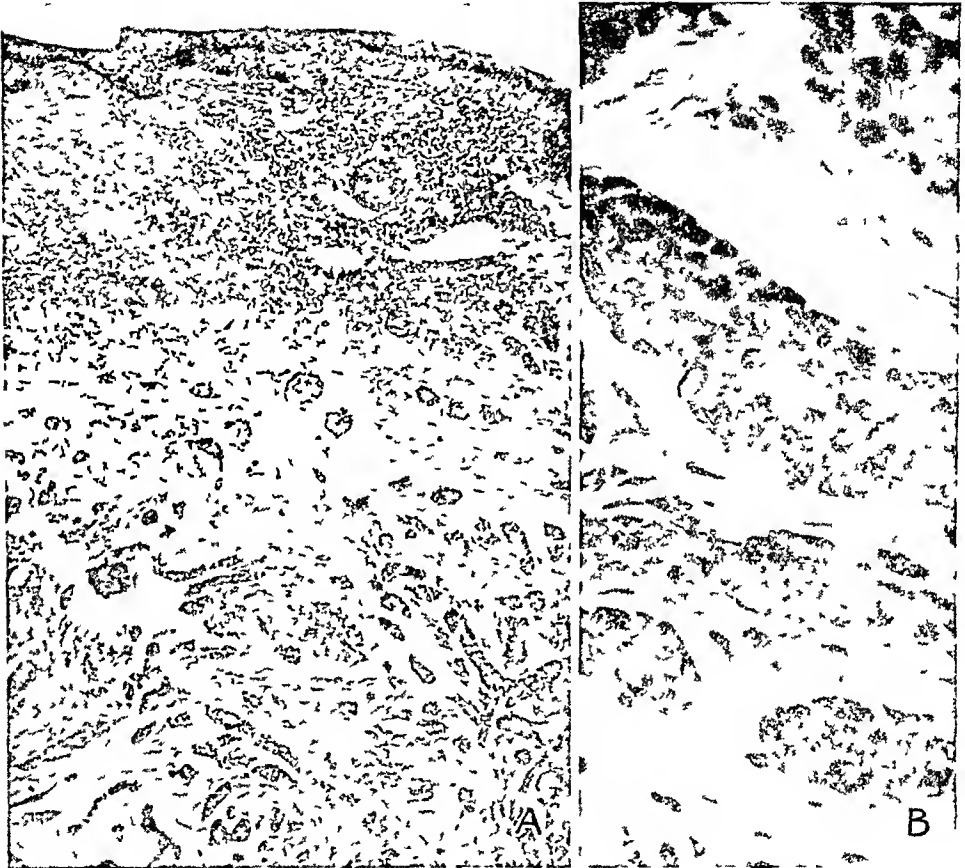


Fig 1 (case 1)—A, biopsy specimen of ear showing invading basal cell masses beneath intact epidermis,  $\times 40$  B, higher magnification of an area of A showing uniform hyperchromatic basal cells,  $\times 300$

after the tumor first appeared in the skin of the forehead. Autopsy showed a neoplasm typical of basal cell carcinoma, metastatic to bones and lungs.

Two instances were reported by Spies.<sup>8</sup> One patient was a 58 year old white man, who had a 2 mm tumor in the right nasolabial fold, twice treated with radium, but recurrent again. On surgical removal it proved to be basal cell carcinoma. It recurred three months later, and at autopsy metastases were found in ribs, spleen and liver. The second patient, a 50 year old white woman, had a tumor of the nasal mucosa, which gave rise to an epigastric metastasis diagnosed as basal cell carcinoma. At autopsy

7 De Navasquez, S. J. Path. & Bact. 53: 437, 1941

8 Spies, J. W. Arch. Surg. 21: 365 (Sept.) 1930

basal cell growths were found in spleen, peritoneum, lungs, ribs and femur

#### REPORT OF CASES

CASE 1 —J I, a white man 37 years old, was admitted to a hospital, March 4, 1938, with "malignancy of the left ear" In 1931 a "cystlike tumor" of the left



Fig 2 (case 1) —Lung showing tumor metastases

external auditory meatus was removed It was not examined microscopically In 1933 a spreading ulcer developed in the wound After removal of the ulcerous area, the skin was smooth for eighteen months, but in 1935 a new ulcer appeared Two more recurrences followed inadequate removals

In November 1937 he noted numbness and weakness of the left side of the face, with "fulness" in the ear The next month he had vertigo, nausea and

vomiting There was severe pain in the left ear and in the chest He had lost 40 pounds (18.2 Kg) in six months and was weak

Examination revealed a weakened, ill nourished white man The left tragus was missing, the concha and the lobe of the ear were distorted and partly missing, and the auditory meatus was obliterated The tissue near the meatus was indurated and ulcerated Two firm nodes were left 2 cm below the tip of the mastoid process There was complete paralysis of the left side of the face Some generalized abdominal tenderness was present

Biopsy of the ulcerated area showed basal cell carcinoma (fig 1A and B)

Roentgenograms revealed marked increase of density in the left mastoid process, any many rounded densities 1 to 4 cm in diameter in both lung fields

Since basal cell carcinoma is but rarely responsible for visceral metastases, another primary source was sought Physical examination of nasopharynx, mouth, larynx, neck, rectum (by sigmoidoscope) and bladder (by cystoscope) disclosed no tumor Roentgenologic study of the whole digestive system revealed no evidence of involvement Pyelograms showed no renal lesion

Roentgen therapy to the region of the left ear and to the chest produced remission of the nausea and vomiting and the pain, and the patient left the hospital May 11, 1938

Oct 14, 1938 he felt well, he had regained his previous weight, and the ulceration of the external ear was healed, though four fifths of the ear was missing Roentgenograms showed 50 per cent shrinkage of the pulmonary growths

March 10, 1939 he noted pain in the upper abdominal region and a loss of 4 pounds (1.8 Kg) in weight A repetition of the search for a second primary tumor was fruitless

He died Aug 1, 1939, eight years after the first tumor appeared on his left ear

*Autopsy*—The body was somewhat emaciated The left external ear was represented by only a few stubs covered with smooth skin

The pericardium presented on its outer surface a few 1 cm firm white plaques of tumor The heart showed no lesion.

The lungs adhered by a few weak bands to the parietal pleura which, on both lateral and diaphragmatic surfaces, was studded with great numbers of 2 mm to 2 cm white tumor nodules The lungs weighed 1,000 Gm each and displayed numerous masses of pale yellow, slightly spongy tumor, the largest 5 cm in diameter (fig 2) The main bronchus of the upper lobe of the right lung was occluded in its upper part by a granular pale gray mass which was thought at first to be a primary bronchial carcinoma A 1 cm abscess lay just distal to this occlusion The bronchi presented no other lesions The lung tissue proper was edematous

The peritoneum and the mesentery, like the pleura, were the site of tumor nodules up to 2 cm across The intestines appeared unchanged throughout

The liver weighed 3,000 Gm, and on its surface were several tumors, the largest 8 by 6 cm The hepatic tissue was passively congested

In the spleen, which was congested and which weighed 350 Gm, was found a single 1 cm white round tumor The tracheobronchial and periaortic lymph nodes and one small node near the tail of the pancreas were enlarged by firm tumor There were two small accessory spleens

Each kidney contained several tumor nodules, averaging 1 cm in diameter



Since a tumor had been found in one bronchus, it was suspected that all the tumors elsewhere were metastases of that one. Microscopic study, however, led to a different conclusion.

*Histologic Examination*—The tumor in all its locations (fig 3A and B), including the affected bronchus, was composed of invasive groups of small oval cells with pale cytoplasm and very indistinct cell boundaries and with extremely rare mitotic figures. No resemblance to squamous cells or to glands was seen although rounded spaces filled with mucoid material were common. The tumor resembled basal cell carcinoma with cystic degeneration and was similar to the biopsy specimen of the ear taken seventeen months before death, except that the latter was not cystic. The anatomic diagnosis, therefore, was basal cell carcinoma.

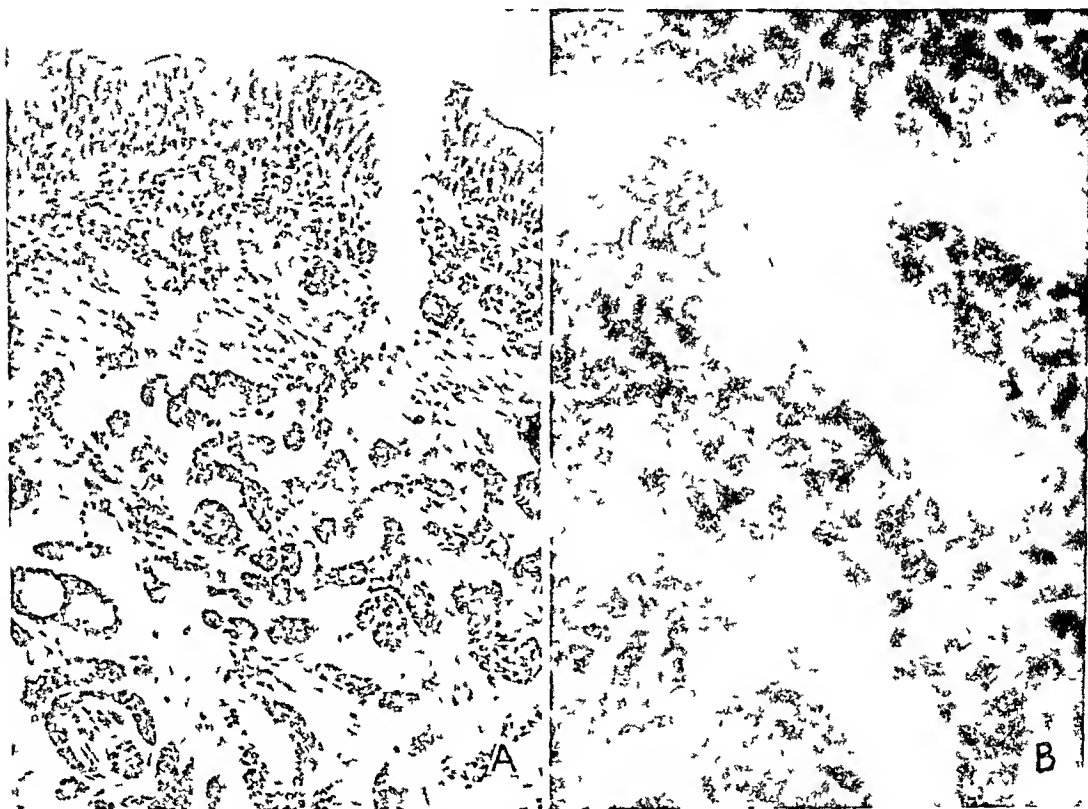


Fig 3 (case 1) —A, bronchus showing tumor in the bronchial wall. Note the cystic formations and the uniformity of the cells,  $\times 60$ . B, higher magnification of an area of A,  $\times 300$ .

of the left ear, pulmonary, hepatic, splenic, renal, pleural, peritoneal, pericardial and lymph node metastases of basal cell carcinoma, pulmonary edema. Permission to open the cranium was not secured.

CASE 2—J. C., a white man aged 62, was seen in the tumor clinic of the San Bernardino County Hospital, Feb. 7, 1938, with a 2 by 3 cm. indurated ulcer of ten years' duration just anterior to and involving the right auditory meatus.

A biopsy specimen of the margin of the ulcer was diagnosed as basal cell carcinoma with some areas suggestive of early squamous cell carcinoma.

March 2 the right ear and the ulcerous area were amputated with the cutting cautery. The specimen showed a 2 by 3 cm ulcer, microscopic study of which revealed basal cell carcinoma with invasion of the perichondrium of the cartilage of the ear.

Since the amount of skin removed from the face precluded primary closure, the wound was left open, and future skin grafting was contemplated. Roentgen therapy, a total of 6,600 roentgens, was given. Although no tumor recurrence was evident, the wound never healed well. Repeated debridement was done, and finally the temporomandibular joint was removed, but healing was never satisfactory.

Jan 28, 1939 the patient was seen in the tumor clinic complaining of pain in the chest and the shoulders, and of cough, but no fever. Roentgenograms showed mottling of both lung fields.

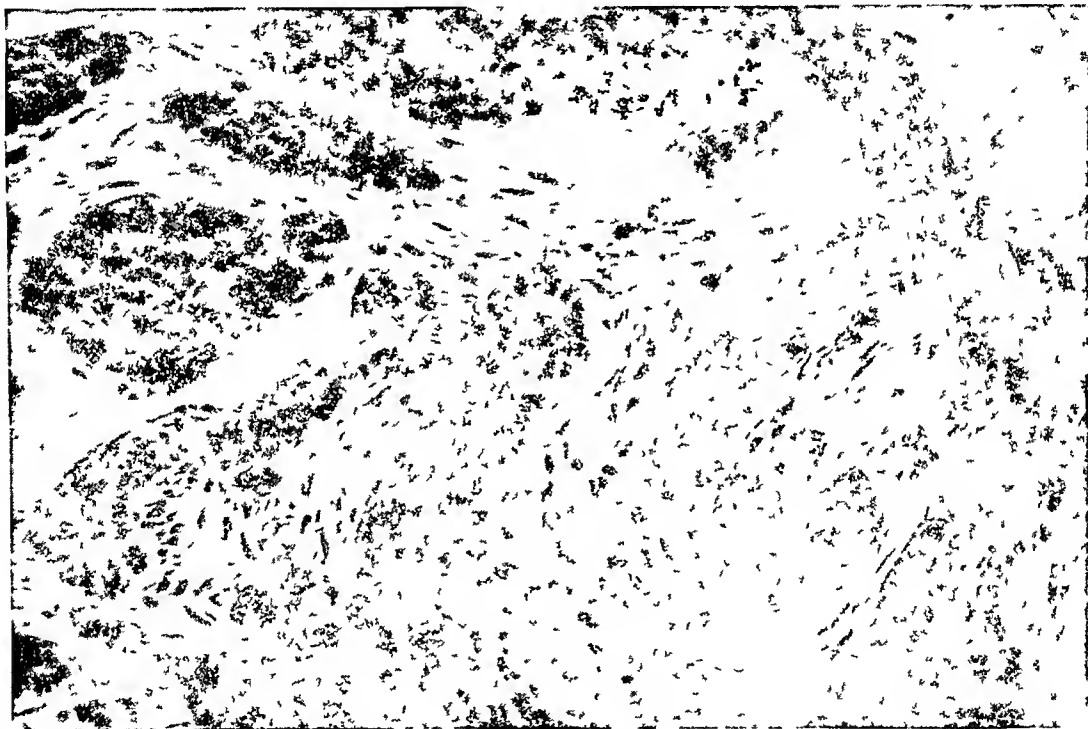


Fig 4 (case 2) —Basal cell carcinoma,  $\times 300$

From this time on he gradually became weak, had persistent pain in the region of the right ear and in the chest, and died Aug 17, 1939.

*Autopsy* (forty hours after death) —The body was that of an emaciated elderly white man. The right ear was completely absent, and a 6 cm ulcer with thick raised margins occupied its site.

The brain weighed 1,400 Gm and contained no lesion. Beneath the dura of the right middle cranial fossa were a few tiny flakes of firm white tissue.

The lungs showed many 2 to 3 mm crumbly white tumor nodules, not in proximity to bronchi. There were also two 2 cm tumor growths near the pleura.

The liver weighed 1,400 Gm and contained about 50 firm white tumor nodules up to 8 mm in diameter. The spleen weighed 100 Gm, was firm and

light red, and contained three white tumor nodules about 7 mm across. The tracheobronchial nodes did not contain grossly obvious tumor, but microscopic sections revealed tumor invasion. No enlargement of the cervical lymph nodes was seen.

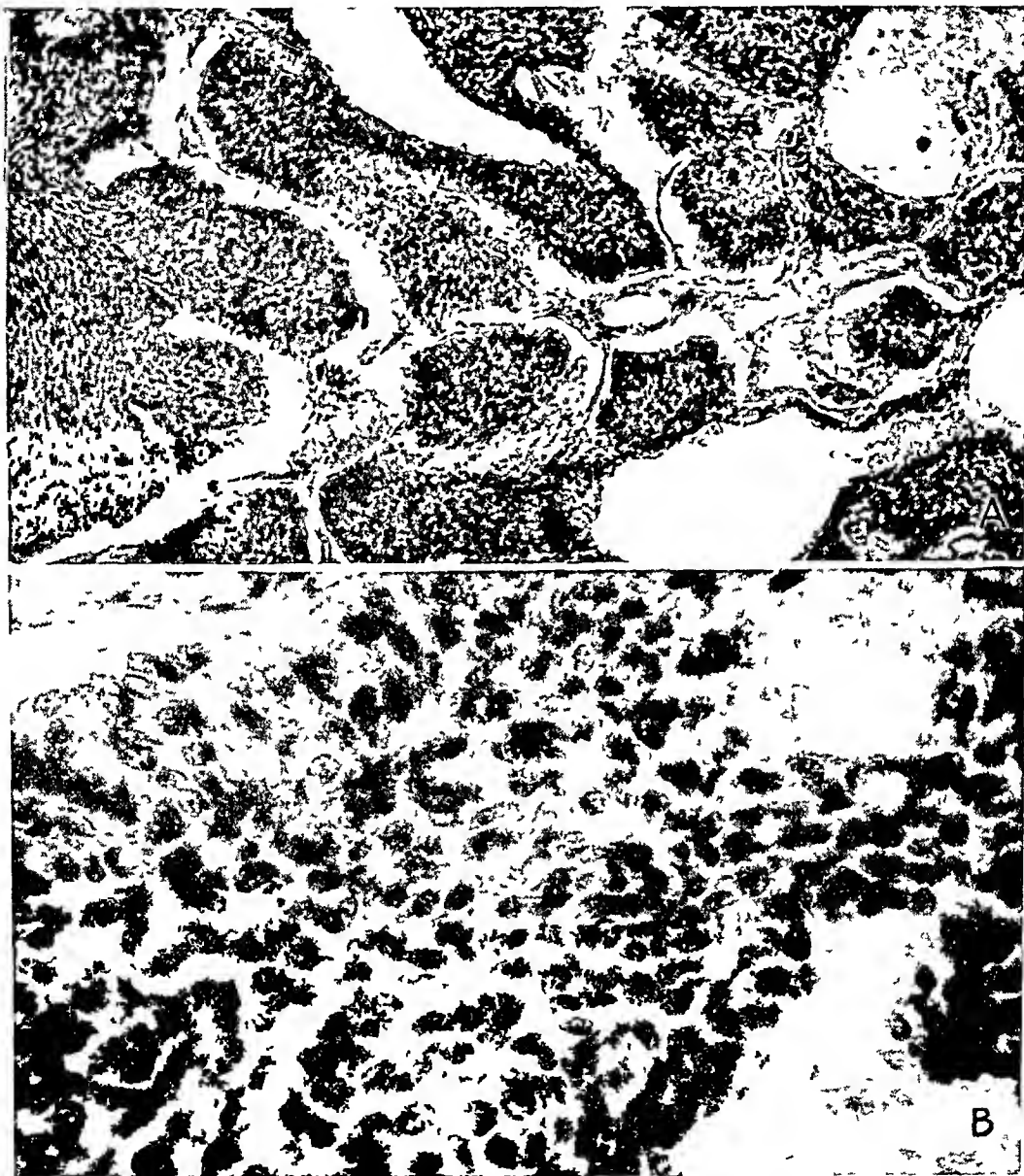


Fig 5 (case 2) —*A*, lung showing intra-alveolar tumor cell invasion,  $\times 80$   
*B*, higher magnification of an area of *A*,  $\times 500$

Each kidney weighed 125 Gm, and each contained a few tumor growths up to 1 cm in diameter. The prostate gland was small and rubbery.

The circulatory and digestive systems revealed no lesion. No tumor or thrombus was seen in the exposed right lateral dural sinus.

*Microscopic Observations*—Reexamination of the original biopsy specimen from the ear confirmed the diagnosis of basal cell carcinoma (fig 4) The squamous cell elements only remotely suggested neoplasia

The tumor growths in lungs, liver, spleen, tracheobronchial lymph nodes and kidneys (fig 5) were composed of sheets and cords of uniform medium-sized oval cells with pale cytoplasm and rather dark nuclei Mitotic figures were scarce The cells were similar to those in the antemortem biopsy specimen The stroma accompanying the tumor was edematous connective tissue In the tumor areas of the lung the alveoli were not displaced, nor were their walls destroyed, they were simply filled with solid sheets of tumor, suggesting that the tumor had penetrated into a bronchus and spread by extension into the alveoli

Sections from the thickened margin of the ulcer of the temporal region, however, revealed quite typical squamous cell carcinoma This recalls the suggestion of squamous cell carcinoma in the original biopsy, though squamous elements were admittedly meager in the biopsy tissue

The anatomic diagnosis was basal cell carcinoma of the ear, squamous cell carcinoma of the skin of the region of the ear, metastases of basal cell carcinoma in the lungs, the liver, the spleen and the kidneys

#### COMMENT

CASE 1—Since the head was not opened, there may be some suspicion that the primary tumor was an intracranial one However, metastases of primary intracranial tumors are rare, hence an intracranial tumor would be improbable as the source of the visceral metastases here Certainly there was some lesion of the vestibular apparatus to account for the vertigo, which was severe enough to prevent the patient's sitting up What this lesion was we unfortunately do not know That the tumor of the external ear had either arisen in or extended to the canal of the ear was shown in the scarring and deformity of that region and in the biopsy specimen obtained on the first clinic visit, part of which came from the canal of the ear The fact that vertigo, nausea and vomiting ceased entirely under roentgen therapy, and that the enlargement of the mastoid region, which regressed under roentgen therapy did not return, and that there were no symptoms of intracranial disease during the last sixteen months of life, is against the idea of the presence of an expanding, or even a persisting, organic lesion of the brain or the internal ear Indeed, it may be that the vestibular symptoms occurred because of persistent occlusion of the auditory canal The conclusion, therefore, was that the basal cell carcinoma of the external ear was the primary tumor and gave rise to the other growths

CASE 2—The diagnosis of the original biopsy specimen as predominately basal cell carcinoma seemed to be beyond dispute The extent of participation of typical squamous cells in that specimen is quite small

The principal question to be settled is whether the visceral metastases sprang from a primary tumor arising in a bronchus or from basal cells of the cutaneous tumor Gross examination did not disclose any primary tumor of a bronchus or of any other site

The original biopsy specimen and the visceral metastases were distinctly basal cell type. The tumor taken from the temporal region at autopsy was squamous cell type. However, the fact remains that the metastases still resembled the obviously basal cell tumor of the biopsy and bear little similarity to the temporal tissue removed at autopsy. This squamous cell carcinoma must have developed from squamous cell elements in the original tumor.

#### SUMMARY

Two cases of basal cell carcinoma of the external ear with generalized metastases are presented.

## FORMATION OF HEMOSIDERIN AND HEMATOIDIN AFTER TRAUMATIC AND SPONTANEOUS CEREBRAL HEMORRHAGES

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THE FORMATION of hemosiderin and hematoidin subsequent to injuries and hemorrhages of the brain was studied early by Virchow<sup>1</sup> and later on by Langhans,<sup>2</sup> Quincke,<sup>3</sup> Neumann<sup>4</sup> and Duerck<sup>5</sup>. Since that time the reactions following cerebral injuries and hemorrhages in men and animals have been described by a number of investigators<sup>6</sup>, detailed reports have been given recently by Rand and Courville,<sup>7</sup> Hicks,<sup>8</sup> Baggenstoss, Kernohan and Drapiewski<sup>9</sup> and Tedeschi<sup>10</sup>. Still opinion differs as to the exact time at which histiocytes containing hemosiderin and (or) hematoidin appear after traumatic and spontaneous cerebral hemorrhages. For this reason experiments were made with mice, the results were compared with autopsy observations of man.

### MATERIAL AND METHODS

Under chloroform anesthesia, stabbing wounds of one or both hemispheres were made with a thin needle in 50 white mice. In 11 of these mice two or three punctures were made at different times. The mice were killed at intervals ranging

From the Laboratory of the Metropolitan State Hospital

- 1 Virchow, R. Virchows Arch f path Anat **1** 379, 1847
- 2 Langhans, T. Virchows Arch f path Anat **49** 66, 1870
- 3 Quincke, H. Virchows Arch f path Anat **95** 125, 1884, Deutsches Arch f klin Med **25** 567, 1880, **27** 193, 1880
- 4 Neumann, S. Virchows Arch f path Anat **111** 25, 1888, **177** 401, 1904
- 5 Duerck, H. Virchows Arch f path Anat **130** 29, 1892
- 6 Tschistowitsch, T. Beitr z path Anat u z allg Path **23** 321, 1898  
Cone, W. Arch Neurol & Psychiat **20** 34, 1928 Macklin, C, and Macklin, M  
ibid **3** 353, 1920 Penfield, W. Surg, Gynec & Obst **39** 803, 1934 del Rio  
Hortega, P, and Penfield, W. Bull Johns Hopkins Hosp **41** 278, 1927 Pen-  
field, W, and Buckley, R. Arch Neurol & Psychiat **20** 1, 1928 Russell, D  
Am J Path **5** 451, 1929 Wilson, R. Arch Neurol & Psychiat **15** 75, 1926  
Winkelman, N, and Eckel, J. ibid **31** 956, 1934 Hassin, G. ibid **36** 231, 1946
- 7 Rand, C, and Courville, C. Arch Neurol & Psychiat **22** 738, 1931,  
**27** 605 and 1342, 1932, **31** 527, 1934, **36** 1277, 1936, **55** 79, 1946
- 8 Hicks, S P. Arch Path **43** 15, 1947
- 9 Baggenstoss, A, Kernohan, J, and Drapiewski, J. Am J Clin Path  
**13** 333, 1943
- 10 Tedeschi, C. Arch Neurol & Psychiat **53** 333, 1945

from three hours to thirty days after the injury. The head was severed from the neck immediately after death and put into 4 per cent formaldehyde solution for twenty-four to forty-eight hours. Then the brain was removed from the skull, fixed again for some time in formaldehyde solution, embedded, cut and stained. For the iron stain Gomori's modification of Perls's reaction was used<sup>11</sup>. The results were compared with the findings in 40 persons with traumatic and 30 with spontaneous cerebral hemorrhages and (or) thromboses associated with hemorrhages whose brains were examined for the presence of iron-containing and iron-free blood pigment<sup>12</sup>. The duration of the hemorrhage was known in most cases from the history or from the onset of the clinical symptoms. The time of survival varied from thirty-seven hours to several years. All age groups were present. Most of the patients with spontaneous cerebral hemorrhages and thromboses were over 60 years old.

### RESULTS

*Mice*—Nervous disturbances resulting from the stabbing wounds and lasting from nine hours to four days were observed in 3 mice. Interrupted compulsory spinning of the body in a circle and loss of equilibrium were seen. Hemorrhages were found in the cerebellum, the basal ganglions and the cornu ammonis at autopsy. As a rule, the mice, after a short period of unconsciousness or drowsiness, recovered fast and behaved later like normal mice. No infections of the wounds occurred. The tracks of the stabbing wounds were often discovered only after several microscopic sections had been stained for iron. The tracks were small. The traumatic reactions varied considerably in different animals. First, edema occurred around the hemorrhagic area, then proliferation and detachment of vascular cells. Polymorphonuclear leukocytes were rarely seen. The histiocytes originated more often from endothelial and adventitial cells of the blood vessels than from microglia cells migrating to the injured area. The effused red cells had disappeared and had generally been engulfed by histiocytes after four to five days. These histiocytes were of varying size and shape—round, elongated or polygonal. They were found outside of blood vessels or in the walls of blood vessels. There was not much difference between histiocytes filled with hemosiderin and situated in the walls of vessels four days or thirty days after the injury.

Hemosiderin became visible in a few instances after forty-eight hours and regularly after seventy-two hours. The cytoplasm of the histiocytes took a pale bluish stain with Perls's test. After the fourth day the number of histiocytes increased, and the hemosiderin became darker blue, filling the whole cell as a diffuse mass or as an accumulation of granules. In stains other than the specific one for iron it was often difficult to discover the wound track and the histiocytes with hemo-

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11 Perls, M. Virchows Arch f path Anat 39 42, 1867. Gomori, G. Am J Path 12-655, 1936.

12 In most of the cases of traumatic hemorrhages the specimens were observed and collected at autopsies performed by the Office of the Chief Medical Examiner of New York, Dr. Thomas Gonzales, who gave me permission to use his material.

siderin They filled the tracks at any time between the fifth and the thirtieth day (The experiments were not extended over thirty days) In several instances the proliferated blood vessels and the histiocytes had a starlike appearance radiating from the center to the periphery Fine yellow granules of hematoidin were discovered in histiocytes in 1 mouse eleven days after the injury, in a second mouse histiocytes

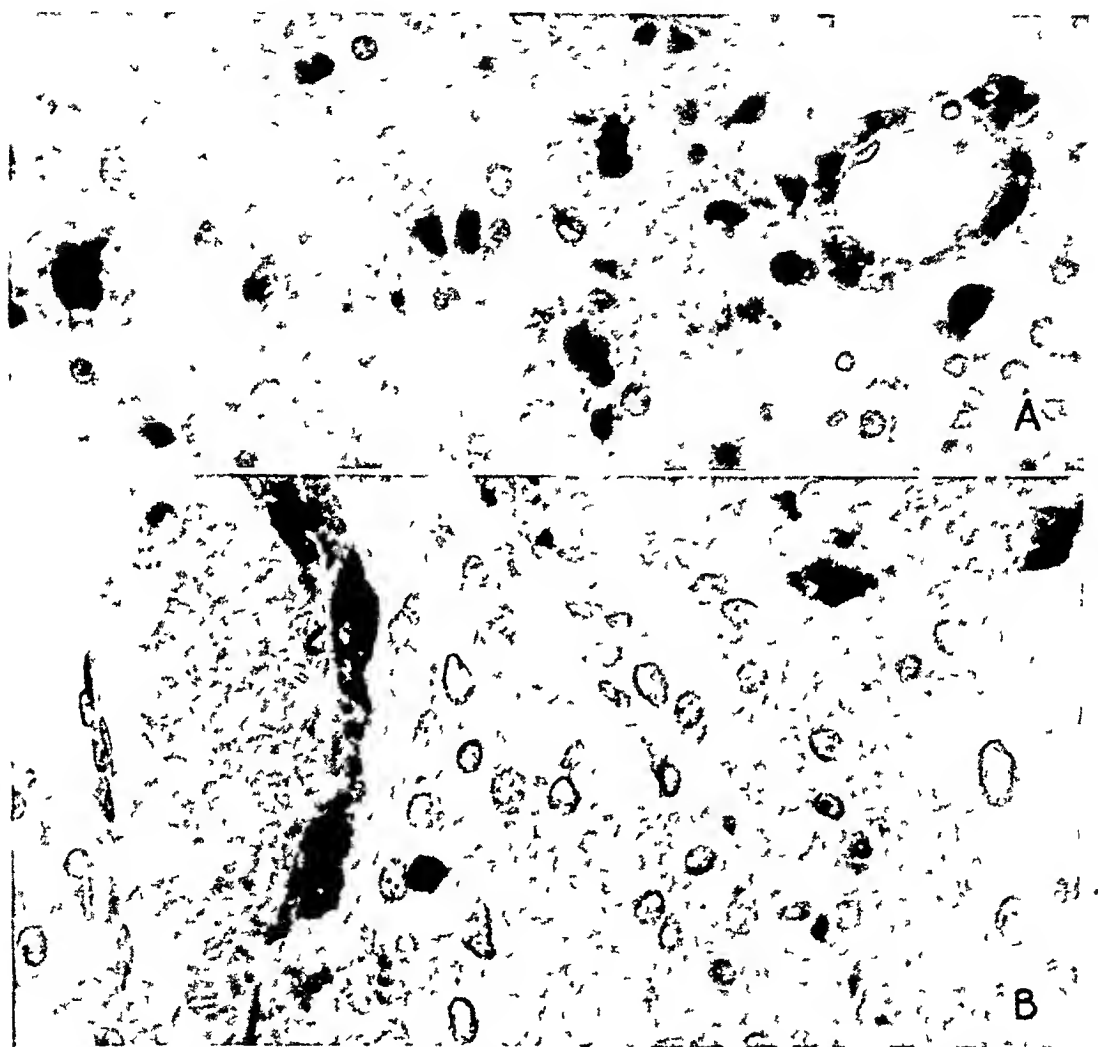


Fig 1—*A*, mouse wound track after four days, showing histiocytes filled with hemosiderin outside of blood vessels and in the walls of blood vessels Hemosiderin stain,  $\times 660$

*B*, mouse wound track after thirty days, showing similar histiocytes filled with hemosiderin around blood vessels and in the walls of vessels Hemosiderin stain,  $\times 660$

with hematoidin and histiocytes with hemosiderin were found in the wound track thirteen days after the injury, in all others the search for hematoidin revealed none Around the wound track after seven days in



several cases there were areas of demyelination and vacuoles, proliferated astrocytes and astrocytic fibrils, and proliferated connective tissue fibers. The nerve cells and the glia cells of the areas adjacent to the wound track appeared normal. Large histiocytes without blood pigment, filled with lipoid material, were also seen.

*Man*—In traumatic and spontaneous cerebral hemorrhages histiocytes containing hemosiderin appeared on the sixth day<sup>13</sup>. These histiocytes had originated from endothelial and adventitial vascular cells and from microglia cells. The number of the histiocytes with hemosiderin increased after this period, and the hemosiderin stained

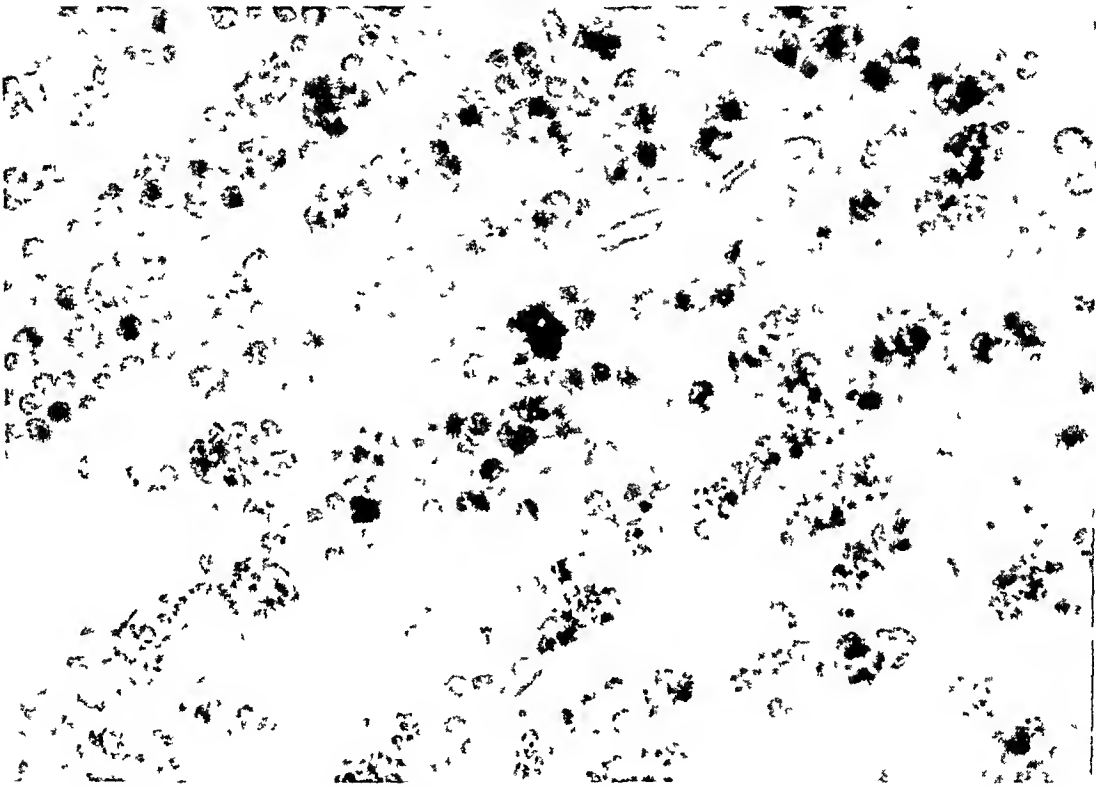


Fig 2—Border of a hemorrhagic area of the brain of a 40 year old man one month after the bleeding occurred. Numerous histiocytes with hematoidin, appearing black in the picture, and other histiocytes with a mixture of pigments or with hematoidin alone may be seen. Hemosiderin stain,  $\times 660$ .

darker blue, some histiocytes invaded perivascular spaces and vascular walls early after the onset of the hemorrhage but most of them remained at the border of the hemorrhagic area for an indefinite period of months and years. Histiocytes containing yellow granules of hematoidin, not giving the iron reaction, became visible in and near the hemorrhagic area ten days after the onset of a spontaneous cerebral hemorrhage in 2 cases. More often hemosiderin was seen in one layer of cells at the

<sup>13</sup> Strassmann, G. Arch Path 38:76, 1944. J Neuropath & Exper Neurol 4:393, 1945.

outer border and hematoidin in an equal number of histiocytes at the inner border after fourteen days in traumatic and spontaneous cerebral hemorrhages. Hematoidin appeared later than hemosiderin. A mixture of both pigments was found in some cells, but these cells were less numerous than the histiocytes containing hemosiderin or those containing hematoidin. In a 40 year old man with hypertension two cerebral hemorrhages were observed. The one had occurred one month before death, the other one, ten days before death. The month-old hemorrhage showed an equal number of histiocytes with hemosiderin and (or) with hematoidin (fig 2). The number of histiocytes with hematoidin was much smaller than the number of histiocytes with hemosiderin in the ten day old hemorrhage.

Generally, besides histiocytes with the blood pigments, numerous histiocytes filled with lipid material were noted in older hemorrhagic areas. Histiocytes with hemosiderin and hematoidin and with lipid stayed in these areas for an indefinite period. But in many cases of smaller cerebral hemorrhages or old subdural hemorrhages and in lobotomy scars of months' and years' duration, only histiocytes with hemosiderin and histiocytes with lipid material were seen. In other cases of older softenings caused by vascular occlusion, no histiocytes with blood pigment and only histiocytes with fatty material were observed.

#### COMMENT

Rich<sup>14</sup> has proved that in tissue cultures mesodermal histiocytes split the hemoglobin from engulfed red cells into an iron-containing compound and the iron-free hematoidin. Muir and Niven,<sup>15</sup> twenty-four hours after injecting blood subcutaneously into rabbits, rats and mice, observed histiocytes containing hemosiderin, histiocytes with hematoidin appeared in rats and mice on the seventh day after the injection. No hematoidin was found in rabbits. In my own experiments<sup>13</sup> alveolar histiocytes containing hemosiderin became visible in rabbits thirty-three hours after intratracheal introduction of blood. In brain injuries of mice histiocytes with hemosiderin appeared occasionally after forty-eight hours and regularly after seventy-two hours. Histiocytes with hematoidin could be discovered in 1 mouse eleven days after the injury. In a second mouse histiocytes with hematoidin and histiocytes with hemosiderin were found in the wound track thirteen days after the injury. Hammes<sup>16</sup> observed histiocytes with iron pigment in subarachnoid hemorrhages of man on the third day. Baggenstoss, Kernohan and Drapiewski<sup>9</sup> saw such histiocytes between the fifth and the seventh day after ventricular punctures. In my own cases of traumatic and

14 Rich, A. R. *Bull. Johns Hopkins Hosp.* **35** 415, 1924.

15 Muir, M., and Niven, J. *J. Path. & Bact.* **41** 177 and 182, 1935.

16 Hammes, E. *Arch. Neurol. & Psychiat.* **52** 505, 1944.

spontaneous cerebral hemorrhages hemosiderin became visible within histiocytes on the sixth day and hematoidin within histiocytes occasionally on the tenth day but more often after fourteen days. The arrangement of two layers of histiocytes, the outer one containing histiocytes with hemosiderin and the inner one histiocytes with hematoidin, near the hemorrhagic area was conspicuous. This fact was stressed as early as 1888 by Neumann<sup>4</sup>. In accordance with Virchow's opinion, it was believed for a long time that only hemosiderin is formed by the action of phagocytic cells and that hematoidin is formed in dead tissue independent of, and distant from, such cells. If hematoidin was observed within cells, it was thought that this iron-free pigment had been engulfed after its extracellular formation<sup>17</sup>. Already in 1884 Quincke doubted that this statement was correct. Since Rich's successful experiments the opinion now is generally accepted that hematoidin, also, is formed by the action of, and within, phagocytic cells. The presence of hematoidin could be demonstrated in histiocytes in our own material ten to fourteen days after the onset of the cerebral hemorrhage. Perhaps the small amount of effused red cells is responsible for the fact that in many instances no hematoidin but only hemosiderin was found. Some pigment may have been dissolved during the embedding process. Histiocytes with hemosiderin were always found earlier after blood was injected into animal tissue (the subcutaneous tissue,<sup>15</sup> the lungs<sup>18</sup> or regional lymph nodes<sup>18</sup>) than after cerebral hemorrhages in man. Apparently, histiocytes were in larger numbers and more easily and faster activated in the animal tissues and organs than in the brain of man. For the same reason histiocytes with blood pigment may appear earlier in subarachnoid hemorrhages than in injuries and hemorrhages of the brain itself, in which the healing process is slow. Two conclusions seem to be justified: (1) Histiocytes with hemosiderin appear on the fifth or sixth day after cerebral injuries and hemorrhages, (2) histiocytes with hemosiderin and histiocytes with hematoidin become visible after ten to fourteen days.

#### SUMMARY

The formation of hemosiderin and hematoidin was studied in injuries of the brains of mice and in traumatic and spontaneous cerebral hemorrhages of man. Hemosiderin appeared earlier after blood was injected into animal tissues or after injuries of the brains of mice than after cerebral hemorrhages in man. It seems probable that scarcity of available histiocytes and their slow activation explain the late formation of the human blood pigments.

17 Hueck, W. Beitr. z. path. Anat. u. z. allg. Path. 54: 68, 1912. Lubarsch, O. Klin. Wchnschr. 4: 2136, 1925. Schmidt, M. B. Ergebn. d. allg. Path. 35: 105, 1940. Virchow<sup>1</sup>. Langhans<sup>2</sup>. Neumann<sup>4</sup>. Duerck<sup>5</sup>.

18 Moritz, A. R. The Pathology of Trauma, Philadelphia, Lea & Febiger, 1942, p. 33.

## SIGNIFICANCE OF EHRlich'S REACTION IN CASES OF MELANURIA

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EHRlich'S test for indole and its substitution derivatives have now been in use for many years, especially in bacteriologic work<sup>1</sup> The test is simple and consists of the addition of an equal volume of Ehrlich's reagent to the solution suspected of containing indole bodies The reaction is also given by solid indole derivatives When indole or its derivatives is present, a "fine red colour"<sup>2</sup> develops in the cold Ehrlich's reagent consists of para-dimethylaminobenzaldehyde, 95 per cent ethyl alcohol and concentrated hydrochloric acid in the proportions 4-380-80<sup>2</sup> The red color is also given by pyrrole, skatole, glucosamine, urobilinogen and notably tryptophane Apparently it is due to the pyrrole ring, though by no means all substituted or condensed pyrrole derivatives react positively Rohde,<sup>3</sup> discussing the reaction with tryptophane in 1905, stated "*Ueber die Natur der farbigen Verbindungen der Aldehyde mit der Skatolaminoessigsäure kann ich heute nur Vermutungen aussprechen*" (Concerning the nature of the colored combining of the aldehyde with the skatole aminoacetic acid I can at present express only a guess) The position has not changed appreciably in the ensuing forty-three years inasmuch as, apart from the probability that quinone formation is involved, nothing more is known of the composition of "rosindole," as the red substance is now called

While it is recognized that normal urines frequently contain indole derivatives in quantities sufficient to give a positive reaction with Ehrlich's reagent, the test is nevertheless often used for the detection of melanuria and is regarded as the most sensitive of such tests

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From the Medical Services, Ministry of Pensions

This work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society

1 Fellers, C R and Clough, R W J Bact **10** 105, 1925 Marshall, W E J Hyg **7** 581, 1907

2 Cole, S W Practical Physiological Chemistry, ed 9, Cambridge, Heffer and Sons, 1933

3 Rohde, E Ztschr f physiol Chem **44** 161, 1905

(According to Snell and Snell,<sup>4</sup> one part of indole per million can be detected by this method) The purpose of this paper is to show that Ehrlich's reaction is a test not for melanin but only for melanogen and that a negative reaction can occur with true melanuria

### EXPERIMENTS

During the course of work on melanin it was desired to investigate the pigment of melanuria. A specimen of urine was obtained from a patient known to have Addison's disease. This gave a strongly positive reaction with Ehrlich's reagent but showed no evidence of melanin formation. The urine was acidified (it was originally faintly alkaline to litmus) and allowed to stand for two days, at the end of which time a deposit of melanin had formed. This deposit was filtered off and, purely fortuitously, the urine again was tested with Ehrlich's reagent. The result was negative. This was attributed to a complete filtering off of the melanin, though that was considered somewhat surprising as it is difficult to remove the whole of this pigment when it is in a state of comparative purity (as it is in melanuria) by simple filtration. Some of the removed melanin was replaced in the urine, but the result was still negative.

#### *Investigation of the Tyrosine-Tyrosinase Reaction by Ehrlich's Test*

| Time | Result of Adding Ehrlich's Reagent |
|------|------------------------------------|
| 2115 | Nil                                |
| 2145 | Nil                                |
| 2215 | Nil                                |
| 2245 | Nil                                |
| 2315 | Very faint pink color              |
| 2345 | Deeper pink                        |
| 0015 | Still deeper pink                  |
| 0045 | Fainter pink                       |
| 0115 | Still fainter pink                 |
| 0200 | Nil                                |
| 0300 | Nil                                |
| 0400 | Nil                                |
| 0600 | Nil                                |
| 0800 | Nil                                |

A fresh solution of para-dimethylaminobenzaldehyde was prepared in alcohol and hydrochloric acid and tested against a standard indole solution (1 cc containing 0.02 mg of indole). A strongly positive reaction occurred. Fresh Addisonian urine was obtained, and again it gave a positive reaction. The urine was acidified and the melanin allowed to deposit for two days as before, but this time the pigment was not removed by filtration. Ehrlich's test was again carried out, and no reaction appeared. The test was then applied to known melanin from a melanoma, to that from the ink sac of *Sepia officinalis* and to the artificial pigment prepared by tyrosinase oxidation of tyrosine. The results, both with solid melanin and with solutions, were uniformly negative.

The oxidizing of tyrosine to melanin by tyrosinase was then followed by means of the Ehrlich test. Two cubic centimeters of a dilute mushroom extract was added to 100 cc of a 0.02 per cent solution of tyrosine buffered to  $p_H$  7.41 (electrometric). The experiment was carried out at room temperature without shaking. Five-tenths cubic centimeter of the mixture was removed periodically and an equal quantity of Ehrlich's reagent added to it. The results obtained are given in the table.

<sup>4</sup> Snell, F. D., and Snell, C. T. *Colorimetric Methods of Analysis*, New York, D. Van Nostrand Co., Inc., 1937, vol. 2.

The reaction became negative again at about the time when a medium brown coloration had developed in the reacting mixture. To the unaided eye melanin formation appeared to be complete by 0400. Obviously a faintly positive reaction might have been masked by the melanin which had formed, although melanin added in varying amounts to a standard indole solution did not hide the color which developed on addition of Ehrlich's reagent. However, the experiment was repeated under the same conditions except that the reaction mixture was filtered before Ehrlich's reagent was added, and, to aid observation, the test was carried out on a white spotting tile. The result was the same. The experiment was again repeated, this time with a 1 per cent solution of tyramine hydrochloride, which is more readily soluble than tyrosine, to increase the probability of a faint positive reaction being observed. Once more the reaction, after becoming positive, became negative again shortly after melanin formation was evident.

There are two obvious explanations of these findings (1) that melanin is not a polymerized indole derivative as is generally assumed, and (2) that polymerized indole derivatives do not give a positive reaction with Ehrlich's reagent. At present there does not appear to be any possibility of deciding which, if either, of these alternatives is correct, as no authentic indole polymers are known to which the Ehrlich test can be applied for comparison. Recent work by Mason<sup>5</sup> supports the view that melanin is an indole polymer.

The details of the oxidizing of tyrosine to melanin, as far as they are known, have been established by Raper<sup>6</sup> and are, briefly, as follows: tyrosine (para-hydroxyphenylalanine) takes up oxygen with the formation of 3,4-dihydroxyphenylalanine (dopa) which is then further oxidized to the orthoquinone. The next step is molecular rearrangement with formation of the indole ring. This indole derivative (5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid) is also converted into the corresponding orthoquinone, which in its turn passes through either 5,6-dihydroxyindole or 5,6-dihydroxyindole-2-carboxylic acid to melanin. The final stages of this reaction and the nature of the pigment itself are still unknown. It appears that the most probable explanation of the behavior of the Ehrlich test during this reaction is that it becomes positive with the formation of 5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid and that it continues to give positive reactions until the actual formation of the melanin, as all the aforementioned intermediate metabolites (with the exception of the second quinone, which is red) are colorless, and that it does not give a negative result again until melanin formation is definite to the unaided eye.

The immediate clinical importance of these findings is that the Ehrlich reagent is a test for melanogen in urine but not for melanin. It follows that a urine which shows a dark brown deposit and which does not give a positive reaction with Ehrlich's reagent may yet be

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5 Mason, H. S. *J. Biol. Chem.* **172** 83, 1948.

6 Raper, H. S. *Biochem. J.* **20** 735, 1926.

that of a case of true melanuria in which the indole derivatives (melanogens) have all been oxidized to melanin. This may well happen with acid urines, especially with specimens sent through the post, i. e., where there is delay in examination.

#### SUMMARY

Evidence is produced to show that Ehrlich's reagent (para-dimethylaminobenzaldehyde) is not a test for melanin but is a test only for the propigment.

This work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society.

# NONPARASITIC, NONCANCEROUS CYSTIC TUMORS OF THE SPLEEN

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ALTHOUGH cystic tumors of the spleen are rare, they are more common than the solid variety. Cystic tumors are less likely to be reported in the medical literature than solid tumors, possibly because the former are usually benign and often their cellular derivation is obvious. There are, however, some reports of cancerous cystic tumors of the spleen and some references to cystic tumors in which the cellular derivation is obscure.

A general survey of cystic tumors of the spleen was presented by Fowler,<sup>1a</sup> who classified them into two large groups: (1) the "true cysts," which included those tumors having a demonstrable cellular lining membrane, and (2) the "false" or "pseudocysts," which included all cysts devoid of a cellular lining layer. In the former group the cellular origin of the tumor is easily determined, while in the latter group no classification based on cellular origin is possible. Fowler<sup>1b</sup> recognized the weakness of the original term "pseudocyst" and subsequently adopted the term "secondary cyst."

The cells involved in the formation of most of the primary cystic splenic tumors are obvious. The group which needs the greatest clarification is the group of so-called epidermoid cystic neoplasms. In instances where elements of ectodermal origin are evident—e.g., hair, keratin and sebaceous glands—the classification is clearcut, and these tumors are properly called splenic dermoid tumors. A more difficult problem of classification occurs in those cases in which the cysts are lined by several layers of squamous cells which often possess intercellular bridges but are without keratinization or epidermal appendages. These tumors are generally known as "epidermoid" cysts and constitute about 10 per cent of all nonparasitic cystic tumors of the spleen. They are

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1 Fowler, R. H. (a) *Ann Surg* 57:658, 1913, (b) *Internat Abstr Surg* 70:213, 1940



probably more frequent than has been generally recognized Custer<sup>2</sup> found 5 in 5,000 autopsies, and we have seen 2 in addition to the one reported in this paper

Within a period of five years the authors have observed 3 cases of splenic tumors, in each of which there was presented a palpable upper abdominal mass and other interesting clinical and pathologic features After complete clinical study, all 3 cases were diagnosed as probable instances of splenic tumors—cystic tumor being considered most likely in 2 of the cases

#### REPORT OF CASES

CASE 1—The patient's past history and the family history were irrelevant The present illness began five years before entry While the patient was exercising at school a slight pain developed in the lower part of the abdomen, which gradually over a period of several hours became quite severe A physician was consulted, who discovered an enlarged spleen but did not make a definitive diagnosis Subsequently there was noted residual mild discomfort, but only rarely did the patient experience severe pain Occasionally he noticed pain in the left shoulder and some fulness in the upper part of the abdomen There was no past history of jaundice, malaria or pertinent infectious diseases He had always lived in the San Francisco metropolitan area

The patient was a thin, underdeveloped young man, aged 20 The only abnormal findings were limited to the abdomen, where there was a prominent bulge and mass in the left upper quadrant, which moved with respiration On palpation the mass was noted to be firm, nontender, round and smooth The tumor extended from the ninth rib superiorly at the midclavicular line to the pelvic brim inferiorly, and laterally from the umbilicus to the flank No other organs or masses were palpated

Examination of the blood gave the following data hemoglobin, 88 per cent (12.1 Gm), red cells, 5,200,000, white cells, 5,400, with polymorphonuclear neutrophils 57, eosinophils 2, lymphocytes 26 and monocytes 15 per cent, platelets, 350,000 sedimentation rate (Wintrobe) 6 mm in one hour, bleeding time (Duke), 2 minutes, clotting time (Lee and White), 3 minutes, fragility of red cells, 0.42 to 0.32, volume index, 0.949, color index, 0.791 Bone marrow obtained by puncture showed percentages as follows myeloblasts, 0.6, promyelocytes, 11.2, neutrophilic myelocytes, 4.6, eosinophilic myelocytes, 7.6, nonfilamented polymorphonuclear neutrophilic leukocytes, 35, filamented polymorphonuclear neutrophilic leukocytes, 4.0, eosinophilic polymorphonuclear neutrophilic leukocytes, 1.6, lymphocytes, 11, monocytes, 5, plasma cells, 1, proerythroblasts, 2.4, erythroblasts, 1.6 Icterus indexes were 5 to 10 The Wassermann and Kahn tests were negative The urine was normal The bengal rose dye excretion test showed 60 per cent retention at 8 minutes and 42 per cent retention at 16 minutes The intravenous hippuric acid test—one hour urine sample, 460 cc—showed 0.86 Gm of hippuric acid The sputum was normal No acid-fast bacteria were found

The clinical diagnosis was splenomegaly, probably cystic

A left paracostal incision revealed an oval, enlarged, apparently cystic spleen which was practically free of adhesions and was easily removed The liver and the gallbladder were grossly normal The patient made an uneventful recovery

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2 Custer, R. P., in Brennemann, J. Practice of Pediatrics, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 3, chap. 20

*Pathologic Report*—The specimen consisted of a spleen measuring 22 by 10 by 6 cm and weighing 1,265 Gm (fig 1). It was grossly lobular because of the presence of six irregular cysts varying from 4 to 12 cm in diameter. The surface was blue-gray except over the cysts, where the pale connective tissue was evident. When the largest cyst was opened, 580 cc of thick, brown-colored fluid filled with cholesterol crystals was released. The lining of this cyst and of the smaller ones was smooth, glistening and characterized by trabeculation. Around the larger cysts there were small satellite cysts and focal areas of fibrosis.



Fig 1 (case 1)—Multiple separate cysts are evident in a spleen weighing 1,265 Gm. The largest one has been drained of its fluid, and the coarsely trabeculated wall is seen. In the smaller cysts the fluid has solidified after fixation.

The cysts were lined by squamous cells, many of which were swollen and cuboidal (fig 2). Frequently, these cells were arranged in double or triple layers. At a few points, six or seven layers of squamous cells were noted. There was no keratinization, but some of the cells had distinct intercellular bridges. Hairs and glandular elements were absent. The cysts were either empty or filled with an amorphous pink-staining material containing a few macrophages.

Beneath the cyst wall there was a variable amount of connective tissue which merged gradually into the surrounding splenic tissue. In the splenic tissue the malpighian bodies were well formed, not hypertrophied, and contained normal central arterioles. The pulp was normal except for increased deposition of inter-sinusoidal connective tissue.

*Diagnosis*—Multiple metaplastic mesodermal ("epidermoid") cysts of the spleen.

**CASE 2**—A 26 year old married white woman complained of a mass in the left upper quadrant of the abdomen, which was moderately tender. The family history was irrelevant. Five years before the present entry a diagnosis of "abdominal

cyst" was made, and on surgical intervention an enlarged spleen and three cysts were encountered. One cyst was located in the right ovary, and two were joined together above, and not attached to, the ovary. These cysts were removed, and the patient was told that they weighed 13 pounds (about 6 kilograms). No further detail concerning the cysts or the spleen was available. Following the operation she had been well except for some soreness of the left upper abdominal quadrant and evidences of a gradually enlarging mass in that region. One month before the present entry, while dancing, the patient first noted a rapidly enlarging lump in the base of the left side of the neck. It grew to 3 cm in size and then remained

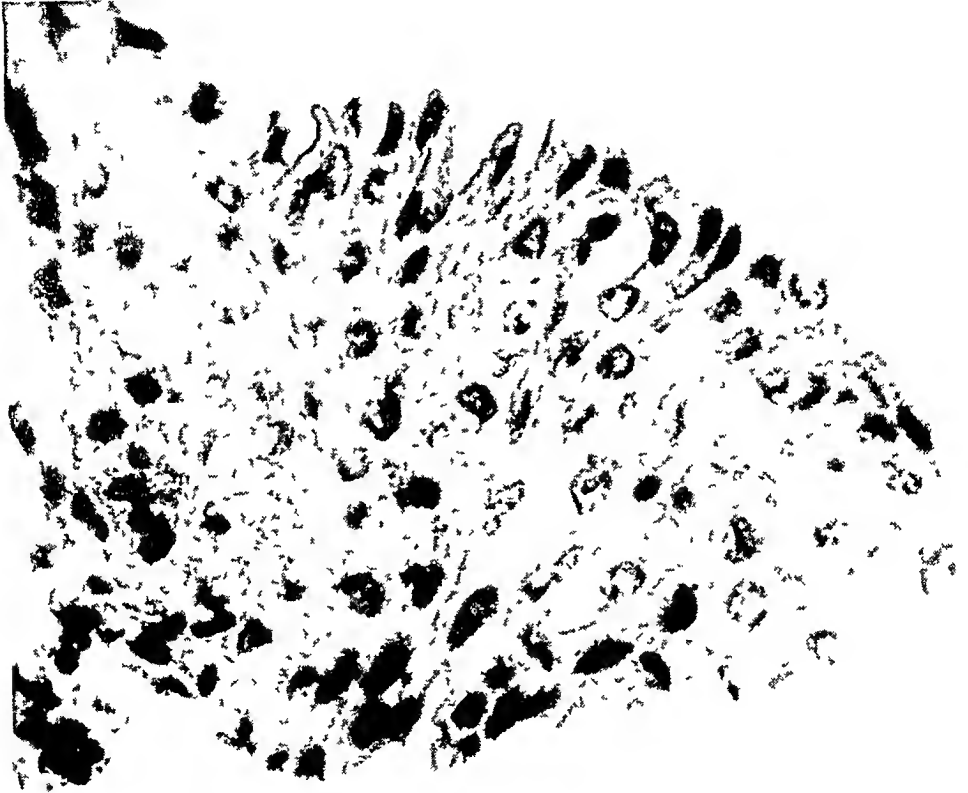


Fig 2 (case 1) —High magnification of a portion of the multilayered squamous cell lining of one of the cysts. Intercellular bridges are present, keratinization is absent.

unaltered to the time of examination, a period of three weeks. In addition, the patient had a small red hemorrhagic tumor of the left index finger, which she stated became enlarged with each menstrual period and then regressed.

The pertinent findings were limited to the abdomen and the neck. The spleen was felt 13 cm below the left costal margin and extended to the midline, its surface was coarsely nodular, and it was tender at the inferior pole. The liver was not enlarged. No other masses or abdominal abnormalities were noted. In the left cervical region, to the right of the insertion of the sternocleidomastoid muscle, was a soft, rubbery, easily movable mass measuring about 3 cm in diameter.

The hemoglobin was 74 per cent (10.2 Gm). The blood counts were: red cells, 4,610,000; white cells, 9,500, with polymorphonuclear neutrophils, filamented,

49 per cent, nonfilamented, 13 per cent, eosinophils 4 per cent, lymphocytes 15 per cent, and monocytes 19 per cent, platelets, 360,000 The Wassermann and Kahn tests were negative The urine was normal The bengal rose dye excretion test showed 58 per cent retention in 8 minutes, and 30 per cent retention in 16 minutes Echinococcus antigen and human and bovine tuberculin tests gave negative results The dextrose tolerance test with insulin was normal

The mass in the cervical region was removed surgically, and the pathologic diagnosis was cavernous lymphangioma (cystic hygroma) One month later splenectomy was performed At this operation, exploration revealed that the liver and gallbladder were grossly normal, that both ovaries were present and that the left contained a cystic tumor, 5 cm in diameter

*Pathologic Report*—The specimen consisted of a spleen weighing 2,120 Gm and measuring 26 by 20 by 9 cm It was deep reddish purple and studded with many cystic structures, varying from 0.5 to 4 cm in diameter Some of the cysts were tense, others fluctuant, and when the organ was sectioned, they were seen to contain a sticky, albuminous fluid which occasionally was hemorrhagic The cysts were smooth lined, and some were multiloculated

The greater part of each section consisted of small to rather large, frequently conglomerate, cystic structures, replacing and distorting a background of normal splenic tissue Some of these spaces were empty, more of them contained a pale pink serum-like material A few were filled with blood, but inasmuch as many spaces contained only serous fluid it is probable that the presence of blood was an artefact The cysts were lined with a very flat endothelium, and just beneath this was a fibrous wall of variable thickness which merged with the adjacent splenic tissue

*Diagnosis*—Cystic lymphangioma of the spleen

Case 3—A 31 year old single white woman complained of soreness and swelling of the left upper quadrant of the abdomen of six years' duration The family history and the patient's past history are irrelevant The only history of trauma was that of a broken ankle which she had at 14 At 15 she had a spontaneous attack of "pleurisy" with pain of the left upper quadrant Movement of any type produced a pain on her left side On the advice of a physician she stayed in bed for two weeks, during which time her temperature rose to 101 to 102 F, and a friction rub was detected over the spleen Since then she has had bouts of aching of the left shoulder and soreness of the left upper quadrant of the abdomen Two years later another physician noted that her spleen was enlarged She was treated medically, without relief

The significant findings were limited to the abdomen The liver was palpable and of normal size A mass in the left upper quadrant, considered to be the spleen, extended 2 cm to the right of the midline and 8 cm below the left costal margin It had a rounded edge, was not tender, had one prominent boss, and was thought to be attached to the diaphragm

The hemoglobin was 90 per cent (12.3 Gm), the blood counts were red cells, 4,800,000 and white cells 7,900, with polymorphonuclear neutrophils 76, lymphocytes 16 and monocytes 8 per cent The platelet count was 380,000 Red cell fragility was normal The icterus index was 5 The bengal rose dye excretion test revealed 50 per cent retention in 8 minutes and 34 per cent retention in 16 minutes The dextrose tolerance test with insulin gave a normal result The Kahn test was negative

The clinical diagnosis was splenomegaly, possible congenital cystic disease of the spleen

A high left rectus incision revealed a greatly enlarged spleen that was adherent to the abdominal wall. It was removed intact. Examination of the liver showed it to be normal. Postoperative recovery was entirely uneventful.

*Pathologic Report*—The specimen consisted of a spleen weighing 1,640 Gm and measuring 18 by 14 by 12 cm. One side of the organ appeared normal. At the lower pole the spleen merged into and formed part of the wall of a large single cyst, the interior of which was fairly smooth and contained turbid brown fluid exhibiting a shimmering sparkle due to cholesterol crystals. The wall was 3 to 6 mm thick and was firm and fibrous.

There was no cellular layer lining the cyst wall, which was composed of dense connective tissue. The inner surface showed degeneration and scattered patches of adherent macrophages. In the cavity of the cyst, amorphous eosinophilic material and cholesterol crystals were encountered. Behind the dense, thick connective tissue wall the splenic pulp was rather fibrous. The malpighian bodies were not particularly prominent, the blood vessels were normal. No areas of deposition of iron pigment were seen.

*Diagnosis*—Solitary secondary cyst of the spleen.

#### COMMENT

The incidence of secondary cysts of the spleen is about four times that of primary cysts. Tumors of angiomatous origin constitute about 65 per cent of the latter. The remainder comprises the epidermoid and dermoid cysts. The dermoid cyst is rare but easily identified. It must be at least partially lined with stratified squamous epithelium and reveal frank keratinization or epidermal appendages (e g, hair or sebaceous or sweat glands). Such a dermoid cyst is exceedingly rare, only 2 instances having been reported (Kumaris<sup>3</sup> and Andral<sup>4</sup>). A third instance of possible splenic dermoid cyst, found in the mesentery at the splenic hilus, has been reported by Velasco Suarez and Angel Etcheverry.<sup>5</sup>

The identification of epidermoid cyst of the spleen is more difficult. It must be at least partially lined with stratified squamous epithelium. The difficulty of classification lies in determining whether the observed lining of squamous cells is true epithelium rather than transformed mesothelium or endothelium. The distinction cannot always be made. However, there may be present certain identifying characteristics, which should be evaluated in order to give accuracy to the reported incidence of true splenic cysts derived from epithelium.

The ontogeny of the spleen offers little aid in the problem, except to emphasize that true epithelium is rarely involved in the development of the spleen. In the early development of the mesogastrium, the splenic

3 Kumaris, J. Arch f klin Chir **106** 699, 1915

4 Andral, G. Precis d'anatomie pathologique, Bruxelles, A. Wahlen et Cie 1837, p 432

5 Velasco Suarez, C, and Angel Etcheverry, M. Arch argent de enferm d ap digest v de la nutricion **12** 168, 1936-1937

anlage is seen to arise from mesenchymal cells as well as from mesothelial cells of the overlying peritoneal surface. The contribution to the splenic parenchyma of the mesothelial peritoneum is rather limited, the major portion being derived from mesenchyme. Some of the early embryologists believed that portions of the spleen arose from cells given off by the nearby pancreatic epithelium, but the more recent work of Thiel and Downey<sup>6</sup> dismissed the possibility of an endodermal contribution to the development of the normal mammalian spleen. In regard of the wolffian bodies, they are spatially too remote to be considered as sources of cystic anomalies of the spleen, although Santy<sup>7</sup> has advanced such an hypothesis. Because of the prominence of mesenchymal cells in the normal development of the spleen, the proof that a primary tumor originated from epithelium must be given with great care, and must overshadow any possibility of the tumor's having been derived from mesenchyme.

An occasional cluster of multiple-layered cells is not a sufficiently distinguishing characteristic to differentiate between an epithelial and a mesothelial (or endothelial) origin of a cyst. This is especially true because much of the reported stratification occurs in crevices and recesses within the cyst wall, and these may be fortuitous. In regard to intercellular bridges, they are suggestive of ectoderm, but Heidenham<sup>8</sup> reported their presence in mesodermal lining cells. If, in addition to stratification and the presence of intercellular bridges, keratinization occurs, the point in favor of a true epithelial origin becomes quite convincing. Lereboullet, Gregoire, Bernard and Ibarra<sup>9</sup> reported a "granular layer containing eleidin granules" in the cells of their case, but they have been the only ones to find even that suggestion of prekeratotic change. In regard to the significance of associated satellite cysts, their cytologic character should be a major contributing factor in deciding whether the observed stratified cellular lining of a cyst represents simple multiplicity of mesenchymal cell layers or layering of true epithelium.

In the first case reported here, the satellite cysts were clearly mesothelial in type, and it is presumed that the multilayered squamous cells arose from stratification of mesothelial cells. Such an origin without doubt accounts for most of the so-called "epidermoid" cysts. The term "epidermoid" as applied to splenic tumors is often interpreted as implying origin from epithelial precursors, and hence is inaccurate. The

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6 Thiel, G. A., and Downey, Hal. *Am J Anat* 28: 279, 1920-1921.

7 Santy, P. *Lyon chir* 27: 101, 1930.

8 Heidenham, M. *Anat Anz* 8: 404, 1893.

9 Lereboullet, P., Gregoire, R., Bernard, J., and Ibarra, R. *Sang* 13: 853, 1939.

desirable term, although cumbersome, would be "metaplastic epidermoid mesodermal cysts" or, for brevity, metaplastic mesodermal cysts, thus leaving no doubt of their true origin

Case 2, in which the patient suffered from cystic lymphangioma of the spleen, needs no further clarification in regard to the cellular origin of the tumor. It was interesting that there were angiomatous abnormalities in other tumors. The patient had had three large pelvic cysts removed, the origin and structure of which were not determined. In addition, a tumor diagnosed as lymphangiomatous hygroma was removed from her neck, and, finally on her left index finger she had a small hemorrhagic tumor which showed cyclic changes associated with the menstrual periods. These multiple tumors having similar backgrounds suggest that the tumor of the spleen was not a neoplasm in the strict sense, but another focus of a generalized congenital anomaly of abnormal vascular channels—hamartoma.

In case 3 there was revealed a typical example of a secondary cyst of the spleen. Before establishing such a diagnosis, however, one should make every effort to take sections of crevices and diverticula, which frequently show a characteristic lining cellular layer. There were none present in the cyst removed from this patient, instead, dense fibrosis of the wall and a lumen filled with brown sediment containing cholesterol were found. Much of the debris was the residue from old hemorrhages of the cyst.

#### SUMMARY

Over a period of five years, 3 patients with enlargement of the spleen were observed, and in each was found a different type of non-parasitic, noncancerous cystic tumor. A large mass in the left upper quadrant of the abdomen directed attention to the spleen, and complete examination supported the original clinical impression but was insufficient to establish an absolute diagnosis. There were no characteristic hematologic findings. The following tumors were removed:

- 1 An "epidermoid" cyst, better termed a metaplastic epidermoid mesodermal cyst. The literature on the subject is reviewed, and the embryologic derivation of these tumors is discussed. Custom has led to the false labeling of these cysts as "epidermoid" cysts. It is suggested that the term "metaplastic mesodermal cysts" would be embryologically and developmentally the most accurate term for them.

- 2 A diffuse cystic lymphangioma, which was attended by other angiomatous tumors elsewhere.

- 3 A simple secondary cyst of the spleen.

# NUCLEAR SIZE IN TETANIZED AND IN CURARIZED SKELETAL MUSCLE

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**I**N PREVIOUS publications<sup>1</sup> I expressed the view that decrease of interstitial pressure may be responsible for an increase of nuclear size and in this way may initiate amitotic division, according to the fact that nuclei divide when they reach a certain size ("critical phase") My view was based on the reactions of nuclei observed in denervated or mechanically injured skeletal muscle, in amputation neuromas, in nerve transplants, in tissue cultures of nerves and in severed tendons

In the case of skeletal muscle it appears that it is the wasting of sarcoplasm which upsets the pressure equilibrium in the muscle fiber, leading to a relative increase of intranuclear pressure The cutting of perineurium and endoneurium elicits in most cases an enlargement of the nuclei of Schwann cells and of endoneurial fibroblasts However, if prior to the injury the nerves are tightly ligated proximally, the severance is not followed by an increase in nuclear volume It is probable that the maintenance of interstitial pressure prevents enlargement of the nuclei Release of tension by severing tendons frequently causes shortening and widening of the tendon cell nuclei with increase of volume What secondary changes are responsible for the nuclear enlargement is not yet known There may occur an alteration in the permeability of the nuclear membrane and changes by osmosis and imbibition

As already mentioned (1948), securing direct proof for the primary role of changes of tissue pressure appears difficult if not impossible, and it was suggested that variations of the previous experiments<sup>1</sup> might give additional support for the thesis In the present report two new approaches are described

## MATERIAL AND METHODS

The animals used in these experiments were frogs (*rana pipiens*) and white rats The subsarcolemmal nuclei of skeletal muscle are large in frogs, they become only slightly contorted (or not contorted at all) if the muscle is tetanized In the first

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1 Altschul, R Arch Path 34 982, 1942, Rev canad de biol 6 485, 1947, Anat Rec 100 517, 1948



TABLE 1.—*Nuclear Size in Tetanized Muscle*

| Number | Normal Muscle * |                    |                |        | Tetanzed Muscle * |                    |                |          |
|--------|-----------------|--------------------|----------------|--------|-------------------|--------------------|----------------|----------|
|        | Width           | Standard Deviation | Standard Error | Length | Volume            | Standard Deviation | Standard Error | Per Cent |
| 1      | 8 25.2          | 981                | ± 221          | 19 43  | 697 4             | 2 958              | ± 160          | 598 6    |
| 2      | 8 30.3          | 796                | ± 178          | 19 21  | 726 8             | 2 837              | ± 206          | 619 4    |
| 3      | 8 111           | 601                | ± 134          | 20 15  | 698 9             | 2 457              | ± 180          | 598 5    |
| 4      | 7 125           | 714                | ± 190          | 19 86  | 573 0             | 3 306              | ± 200          | 509 3    |
| 5      | 8 742           | 610                | ± 136          | 17 78  | 711 1             | 2 661              | ± 168          | 683 7    |
| 6      | 8 703           | 849                | ± 155          | 19 37  | 732 9             | 2 722              | ± 203          | 532 7    |
| 7      | 8 167           | 804                | ± 117          | 19 05  | 664 9             | 2 793              | ± 132          | 550 3    |
| 8      | 8 082           | 893                | ± 163          | 16 57  | 562 2             | 2 475              | ± 134          | 483 5    |

\* All measurements are given in microns

TABLE 2.—*Nuclear Size in Curarized Muscle*

| Number † | Normal Muscle * |                    |                |        | Curarized Muscle * |                    |                |          |
|----------|-----------------|--------------------|----------------|--------|--------------------|--------------------|----------------|----------|
|          | Width           | Standard Deviation | Standard Error | Length | Volume             | Standard Deviation | Standard Error | Per Cent |
| 9        | 8 132           | 831                | ± 186          | 18 44  | 638 1              | 2 616              | ± 164          | 812 7    |
| 10       | 8 194           | 787                | ± 176          | 19 99  | 702 4              | 2 395              | ± 156          | 824 5    |
| 11       | 8 105           | 707                | ± 158          | 20 44  | 702 6              | 2 722              | ± 154          | 844 2    |
| 12       | 7 919           | 672                | ± 106          | 20 91  | 686 2              | 3 589              | ± 131          | 741 6    |
| 13       | 8 097           | 925                | ± 146          | 22 17  | 700 6              | 3 438              | ± 127          | 757 1    |
| 14       | 8 282           | 571                | ± 128          | 20 06  | 720 0              | 3 038              | ± 192          | 834 5    |
| 15       | 7 876           | 711                | ± 112          | 19 86  | 644 7              | 5 109              | ± 145          | 794 4    |
| 16       | 7 384           | 653                | ± 119          | 21 95  | 660 7              | 3 366              | ± 165          | 764 2    |

\* All measurements are given in microns

† Nos 9 to 11 were curarized by intramuscular and intracardial injection, nos 12 to 16 by intramuscular injection only

series of experiments the brain of the animal was destroyed and then the gastrocnemii were excised. One was fixed immediately in 10 per cent formaldehyde solution, while the other was tetanized by faradic current and immersed in 10 per cent formaldehyde solution, the faradization being continued while the muscle was being fixed, until no more relaxation or contraction could be obtained.

In the second series, the gastrocnemius of one leg of the frog in which the brain had been destroyed was excised and fixed in 10 per cent formaldehyde solution, then the animal was curarized by injecting "intocostrin" (0.75 to 0.8 cc of the solution) intramuscularly and intracardially. When the animal was completely paralyzed, the gastrocnemius of the other leg was dissected out and fixed in 10 per cent formaldehyde solution. All the muscles were embedded in paraffin, cut longitudinally and stained with hematoxylin and eosin.

Similar experiments were also carried out on rat muscles. While the tetanizing yielded results conforming with those of the frog experiments, the curarizing of rats was not successful, for small doses gave incomplete relaxation of muscles and large doses elicited fasciculation. For this reason the curare experiments with rats were abandoned.

In measuring the nuclei, the following procedure was followed: the width and the length of each of 20, 30 or 40 nuclei were determined from each muscle, the widest nuclei being selected for the measurements. This was done with the view that (1) if the widest nuclei were selected, the error of a perspective shortening of the long axis would be of lesser importance, (2) one would be dealing with a more homogeneous group, (3) the wide nuclei being by far the more voluminous, the margin of error might be expected to be smaller. In control measurements nuclei were taken at random, i.e., without regard to their width. In some cases 100 nuclei instead of only 20 to 40 were measured. All measurements were done by one person (Miss A. M. Friesen) using oil immersion and an ocular micrometer. Then the average length and the average width of the nuclei were determined, and the average volume ( $4/3\pi ab^2$ ) established—assuming that these nuclei are spheroids. For control purposes, we established also in a few cases (1) the volume of each nucleus and then the average volume, as well as (2) the average width and length and from them the average volume. The latter was practically identical with that obtained by the first procedure.

#### RESULTS AND COMMENT

The results of the frog experiments are evident from tables 1 and 2. The decrease of tonus and the nuclear enlargement in curarized muscle conform with the findings in denervated muscle. In 5 rats the tetanizing of muscle and the increase of tonus resulted in a decrease of volume in subsarcolemmal muscle nuclei by 4, 4, 5, 17 and 19 per cent, respectively.

Admittedly, the technic involves one major error, namely, the assumption that the cross section of a nucleus is a perfect circle, which it never is and which it rarely approaches. But in injured peripheral nerves (Abercrombie and Johnson<sup>2</sup>, Denny-Brown<sup>3</sup>), in skeletal muscle where the sarcolemma was ruptured (Barer<sup>4</sup>) and in curarized

2 Abercrombie, M., and Johnson, M. L. *J. Anat.* **80** 37, 1946.

3 Denny-Brown, D. *Arch. Neurol. & Psychiat.* **55** 171, 1946.

4 Barer, R. *J. Anat.* **81** 259, 1947.

skeletal muscle (my own observations) the cross section of the nucleus becomes rounder as compared with the nucleus of normal nerve or muscle. Therefore the error in assuming that the cross section is a circle in both the normal and the experimental conditions will influence the results by decreasing and not by creating or even increasing the difference between the muscles of the two sides. That means that the differences are most probably greater than reported in table 2 and in a previous publication (1948), and possibly also greater than in table 1. It could be said that from a statistical point of view the number of measured nuclei is small. This may be true, but it should be pointed out that similar measurements were carried out in 52 other cases, published previously and some more, hitherto unpublished, and in all these cases the conditions of decrease or maintenance of interstitial pressure led to similar results regarding the volume of the nucleus. Moreover, nuclear enlargement has been reported under similar conditions, but without measurements and without regard to tissue pressure by Weber <sup>5</sup> and many others for nuclei of skeletal muscle, and by Masson <sup>6</sup> and by Abercrombie and Johnson <sup>2</sup> for sheath cell nuclei of nerves.

#### SUMMARY

The thesis that changes of extranuclear pressure are followed by changes in nuclear volume receives additional support in two sets of experiments. In the first, muscle tonus was increased by tetanizing and the nuclear volume decreased accordingly, in the second series the volume of nuclei of skeletal muscle increased when muscle tonus decreased under curarizing. The latter results parallel those of muscle denervation.

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5 Weber, O    *Virchows Arch f path Anat* **39** 216, 1867

6 Masson, P    *Am J Path* **8** 367, 1932

# ORGANOID DIFFERENTIATION OF THE FETAL LUNG

A Histologic Study of the Differentiation of Mammalian Fetal Lung in  
Utero and in Transplants

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FOR MORE than one hundred years controversy regarding the ultimate structure of the pulmonary alveolus has waxed and waned. One of the principal issues of disagreement has been the question of whether or not this structure is lined by epithelium. Since an understanding of the genesis of various congenital, inflammatory and neoplastic pulmonary lesions depends on the establishment of this and other facts relating to the normal alveolus, the problem is of more than academic interest.

The older literature on the subject was reviewed by Miller,<sup>1</sup> who favored the concept of a continuous alveolar lining of epithelial cells. Other investigators<sup>2</sup> concluded that the cells which proliferate and line the alveoli in response to injury or to the presence of foreign material are derived from the macrophage system. Still others<sup>3</sup> hypothecate the presence of an alveolar lining comprised of non-nucleated protoplasmic plates or films. Rose<sup>4</sup> enlivened the controversy still further by asserting that he could find no evidence that the alveoli develop as an outgrowth of the bronchial tree. He studied the formation of the alveolar capillary plexus from the mesoderm and regarded it as the essential anatomic and functional unit of the lung, bearing a relationship to the bronchi similar to that borne by the glomerular capillaries to the nephron.

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1 Miller, W. S. *The Lung*, Springfield, Ill., Charles C. Thomas, Publisher, 1937

2 Fried, B. M. *Arch. Path.* **3** 751, 1927, **6** 1008, 1928. Robertson, O. H. *Physiol. Rev.* **21** 112, 1941. Geever, E. F., Neuburger, K. T., and David, C. L. *Am. J. Path.* **19** 913, 1943. Clements, L. P. *Anat. Rec.* **78** 429, 1940. Marshall, A. H. E. *J. Path. & Bact.* **108** 129, 1947.

3 Bensley, R. D., and Bensley, S. H. *Anat. Rec.* **64** 41, 1935. Bensley, S. H., and Groff, M. B. *ibid.* **64** 27, 1935. Cooper, E. R. A. *J. Path. & Bact.* **47** 105, 1938. Stewart, F. W. *Anat. Rec.* **25** 181, 1923. Bremer, J. L. *ibid.* **70** 263, 1938.

4 Rose, S. B. *Arch. Path.* **6** 36, 1928

Palmer,<sup>5</sup> Barnard and Day<sup>6</sup> and Ham and Baldwin<sup>7</sup> have more recently emphasized the participation of the capillaries in the development of the terminal air passages as observed in the human fetus. Their studies indicate that the cells which line the terminal vesicles of the immature lung begin to disappear about the end of the fifth month of fetal life and that the unsheathed capillaries come in direct contact with the alveolar space.

The investigation of this problem has been carried on in the past by microscopic examination of serial sections of the lungs of embryos of increasing age. Such sections have usually been stained with hematoxylin and eosin or prepared by comparable nonspecific procedures. There have been numerous exhaustive analyses of such material from several species, some leading to one conclusion, some to another. Maximow and Bloom<sup>8</sup> have summarized the matter with the statement that "the whole question of the type of cell lining the alveoli and the determination of its epithelial or mesenchymal origin demands a thorough embryologic investigation of the lungs in the later stages of intra-uterine life."

With this background there would appear to be little encouragement for one to reinvestigate the problem by the same methods that have been employed in the past. It was felt, however, that there were at least two promising approaches that had not been adequately explored. One was to investigate the cytochemical characteristics of the differentiating pulmonary blastema in the belief that structural differentiation of primitive cells may be preceded by chemical change. Recognition of such change preparatory to structural differentiation might help to establish whether the epithelial elements of the bronchi and the alveoli are derived from the foregut by an infiltrative proliferation of endodermal cells or whether they represent *in situ* metaplasia of the cells of primitive pulmonary mesenchyme.

Another investigative procedure considered worthy of trial was to employ the method which Greene<sup>9</sup> recently described, by which fetal tissue may be caused to grow and differentiate in a heterotopic environment by transplanting it to the anterior chamber of the eye. In these circumstances, organoid differentiation of fetal tissue takes place independently of the influences of somatic integrity, and any differentiation that occurs must be inherent in the transplanted cells themselves and independent of the extrapulmonary organizers or gradients that affect organoid differentiation in the intact fetus.

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5 Palmer, D. M. *Am. J. Anat.* **58**: 59, 1936.

6 Barnard, W. G., and Day, T. D. *J. Path. & Bact.* **45**: 67, 1937.

7 Ham, A. W., and Baldwin, K. W. *Anat. Rec.* **81**: 363, 1941.

8 Maximow, A. A., and Bloom, W. *Text-Book of Histology*, ed. 4, Philadelphia, W. B. Saunders Company, 1943.

9 Greene, H. S. N. *Cancer Research* **3**: 809, 1943.

## HISTOCHEMICAL INVESTIGATION

In the course of surveying the cytochemical characteristics of fetal lung in respect to the presence or the distribution of nucleoprotein (Feulgen reaction<sup>10</sup>), mucoprotein,<sup>11</sup> fat (sudan IV),<sup>12</sup> alkaline phosphatase<sup>13</sup> and glycogen<sup>14</sup> it became apparent that the appearance and disappearance of intracellular glycogen bear an important relation to alterations of cellular appearance and organization

Uninterrupted series of sections stained with Best's carmine were cut from blocks of fetal lungs (mouse, guinea pig, human) of varying gestational age that had been fixed in Carnoy's or Rossman's fluid. Similar series of sections were cut from blocks of the same lungs that had been fixed in Zenker's fluid or 10 per cent formaldehyde solution and stained with either hematoxylin and eosin or eosin-methylene blue.

There were no fundamental differences in these three species of mammals in respect to the histologic appearance of, or relationships between, the elongating and branching bronchi and the primitive mesenchyme in which organoid development of lungs takes place. No fetus was studied which was so young that the pulmonary blastema was devoid of recognizable bronchial differentiation. Fetuses obtained in the first trimester of pregnancy were most suitable for the study of bronchial development whereas those obtained in the second and third trimesters were used for the investigation of alveolar differentiation.

In fetuses so young that there was as yet no alveolar differentiation the primitive lung was comprised of a branched epithelium-lined tube situated in a solid mass of undifferentiated mesenchyme. In the youngest fetuses the mesenchyme was relatively avascular and composed of loosely arranged undifferentiated tissue having an abundant intercellular fluid except in the immediate vicinity of the differentiating alveoli where the intercellular spaces were reduced and the cells more compactly arranged (fig 9). As the pulmonary blastema of slightly older fetuses became vascularized, the entire mesenchyme became more compact and the looser structure seen in younger embryos disappeared along with the intercellular fluid (fig 10).

## BRONCHIAL DIFFERENTIATION IN UTERO

In series of sections stained by ordinary visualizing methods (hematoxylin and eosin or eosin-methylene blue) the impression was gained that the peripheral extension of the primitive bronchi was accomplished by multiplication of the most distally located epithelial cells which invaded and replaced the adjacent mesenchymal elements (figs 9 and

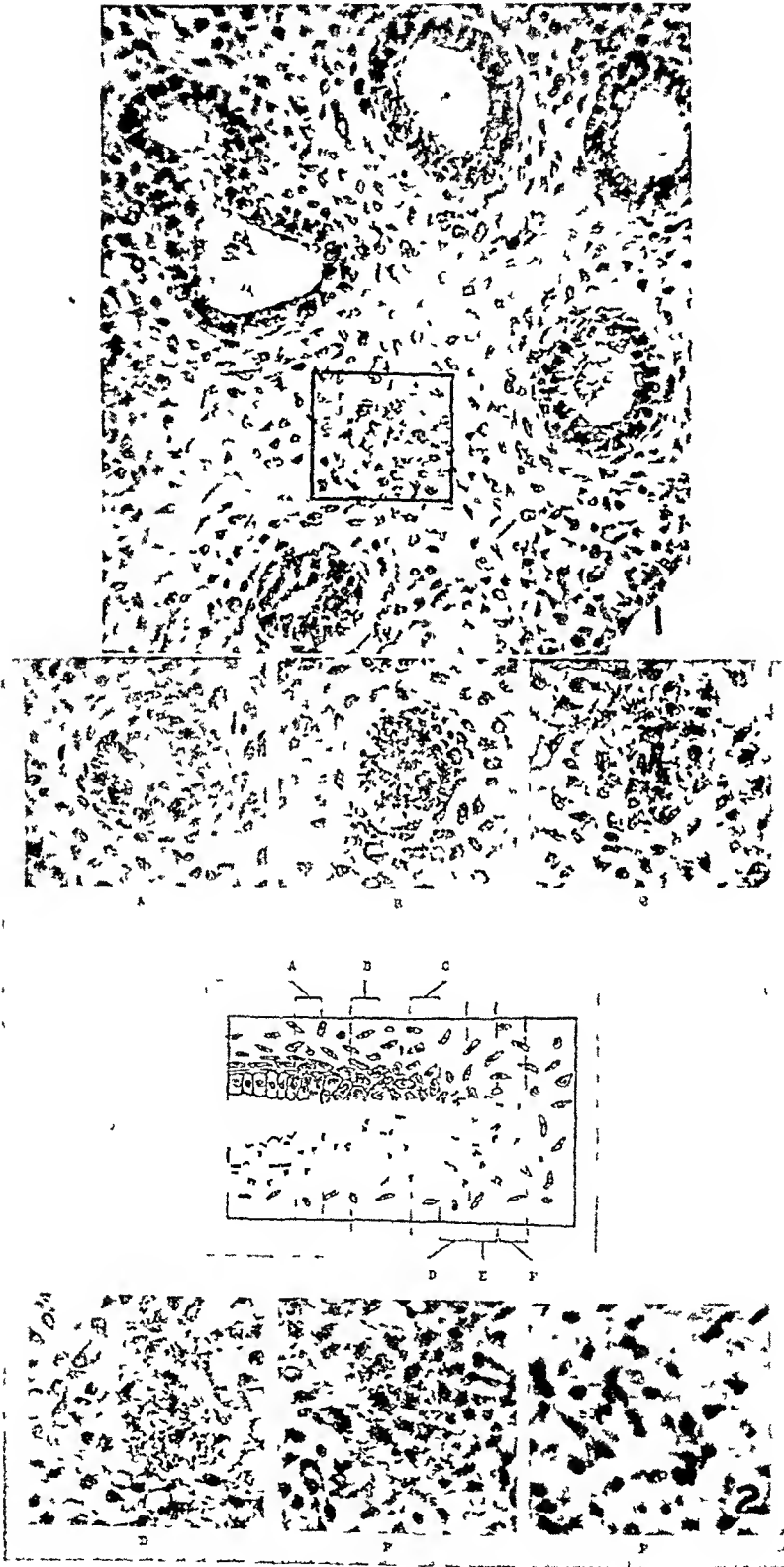
10 Feulgen, R, and Rossenbeck, H. *Ztschr f physiol Chem* **135** 203, 1924

11 Hemplemann, L. H., Jr. *Anat Rec* **78** 197, 1940

12 McClung, C. E. *Handbook of Microscopical Technique*, New York, Paul B. Hoeber, Inc., 1937

13 Gomori, G. *J Cell & Comp Physiol* **17** 71, 1941

14 Benslev, C. M. *Stain Technol* **14** 47, 1939



Figures 1 and 2

10) Thus, it has often been inferred from the study of such preparations that the parenchymatous elements of the pulmonary tissue or at least the bronchial epithelial cells are the direct descendants of the endoderm of the foregut

In series of sections cut from blocks fixed in Carnoy's or Rossman's fluid and stained by the Best carmine technic it became apparent that the bronchial epithelium is probably derived by a quite different process. Peripheral to the growing tip of a bronchus and in continuity with it, a chemical alteration occurs in the primitive cells of the mesenchyme which is the first of a series of changes by which the mesodermal cells are transformed into bronchial epithelium. This chemical change is antecedent to any recognizable alteration of their size, shape or relationship to one another. It consists of an intracytoplasmic accumulation of small droplets of glycogen. These droplets are so small that without special preparation and staining they escape detection, i. e., they are not large enough to give the appearance of vacuolation after they have been removed by solution (figs 2 and 9). This preliminary chemical change which appears to be a precursor of structural differentiation is not peculiar to the transformation by which mesenchyme becomes bronchial structure. It also precedes the metamorphosis by which mesoderm develops as primitive blood vessels (fig 3).

In the case of developing bronchi the glycogen appeared in the cytoplasm of the mesenchymal cells as far as 20 or 30 microns in advance of recognizable structural differentiation (fig 2 *C, D* and *E*). Immediately proximal to this initial chemical alteration the first morphologic evidence of differentiation appeared (fig 2, *B* and *C*). The glycogen-containing cells underwent active mitotic division, became individually swollen and formed a poorly defined but compact cellular island in the otherwise loosely arranged mesenchyme. As the tip of the elongating

Fig 1—Low magnification orienting view of a portion of the lung of a 44 mm guinea pig embryo. In the enclosed area the fine stippling is due to small intracytoplasmic accumulations of glycogen distal to the recognizable tip of a bronchus. Other than this chemical differentiation there is no evidence that this area is proceeding toward bronchus formation. The five larger bronchi show accumulations of glycogen, and in two of them there are small quantities of glycogen which have been extruded into the lumens. The schematic reconstruction of the bronchus shown in figure 2 was made from serial sections passing through this area. Best carmine and hematoxylin,  $\times 225$ .

Fig 2—Semidiagrammatic reconstruction of the tip of a differentiating bronchus of the lung of a 44 mm guinea pig embryo fixed in Carnoy's fluid and stained for glycogen by the Best carmine method. Sections *A, B* and *C* are 10 microns apart, *D, E* and *F* are 5 microns apart. Glycogen is demonstrated in the photomicrographs as darkly stained intracytoplasmic accumulations. It is correspondingly represented by black stippling in the diagram. *C, D* and *E* pass through the zone of chemical differentiation described in the text. There is no morphologic evidence of differentiation here. *B* shows central crowding of nuclei as well as large central accumulations of glycogen. This is the most distal morphologically recognizable part of the bronchus. *A* demonstrates a well formed bronchus with definite epithelium and only a few glycogen droplets. The nuclei are nearer the periphery. Best carmine and hematoxylin,  $\times 870$ .



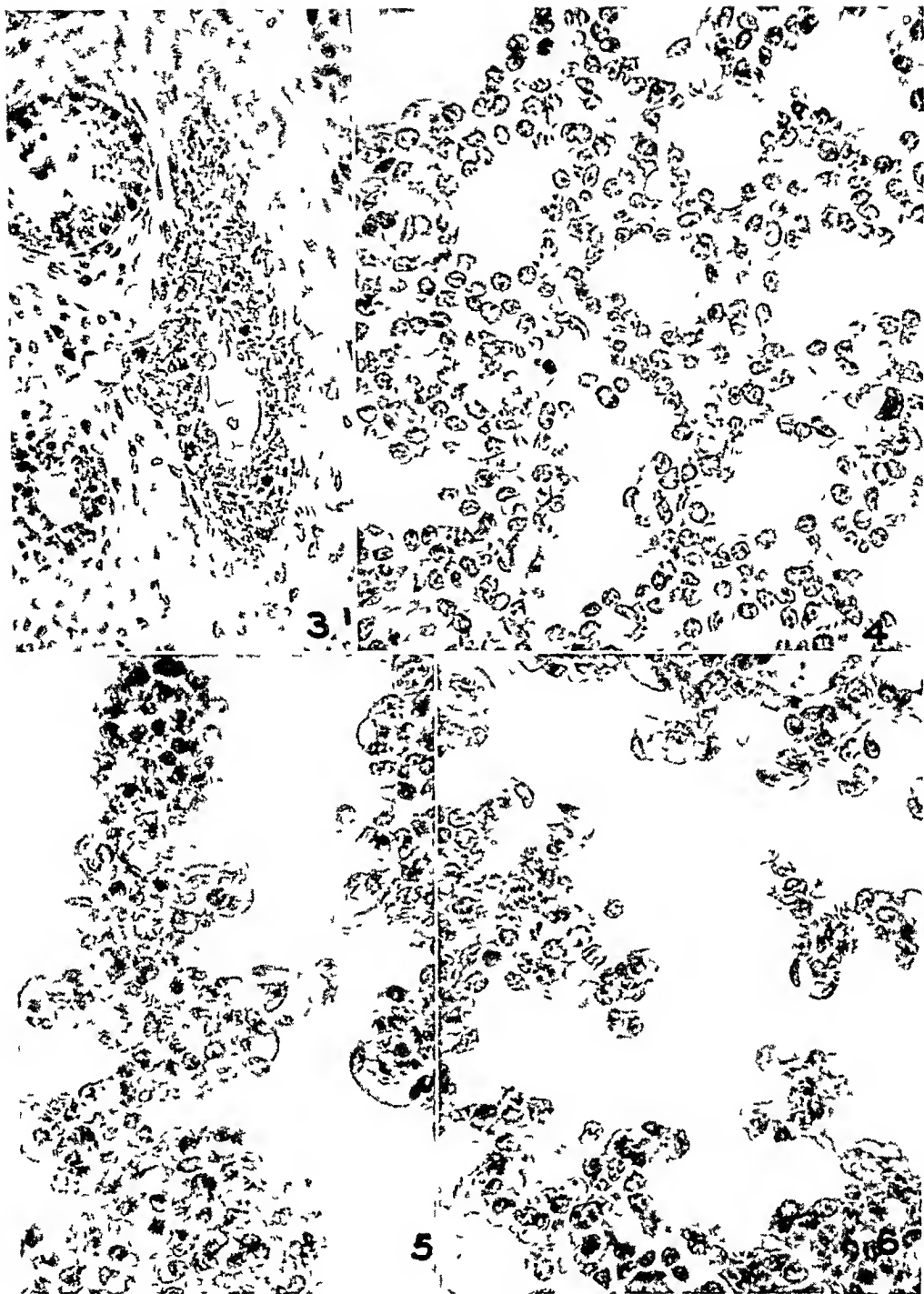


Fig 3—Glycogen accumulating in and about a differentiating arterial wall in the lung of a 44 mm guinea pig embryo. Glycogen is also present in the bronchial epithelium. Best carmine and hematoxylin,  $\times 250$

Fig 4—An early stage of development of a terminal respiratory unit in an 80 mm guinea pig embryo. The parenchyma is still compact, and scattered through it can be seen blood vessels which, although near the lumens, have not come in close contact with them. Notice that not all the cavities have definite epithelial linings. The alining of the mesenchymal cells bordering on these cavities to form epithelium without a basement membrane can be seen. Hematoxylin and eosin,  $\times 425$

Fig 5—Early alveolar formation in the lung of a 95 mm guinea pig embryo. The parenchyma has thinned out and capillary knuckles are pushing into the lumens. Hematoxylin and eosin,  $\times 425$

Fig 6—Further capillary development in the lung of a 105 mm guinea pig embryo. The bulk of the undifferentiated mesenchyma has disappeared, and the number of capillaries has increased. The only visible structure separating the alveolar space from the blood stream is the capillary endothelium. Hematoxylin and eosin,  $\times 425$

bronchus was approached the central cells in such a cluster took on an ill defined radial arrangement and the cytoplasmic inclusions of glycogen increased to such a degree that the cells became coarsely vacuolated (fig 2 C) As yet there was nothing to distinguish these altered mesenchymal cells from their fellows or to indicate that they were to become bronchial epithelium aside from their radial arrangement, their swelling and the increase of their glycogen content As these cells merged with those of the solid tip of the bronchus, the glycogen-containing vacuoles at their outer poles became very large and the nuclei were crowded into a central cluster This resulted in an apparent but not actual local increase in the number of cells (fig 2 B) As observation moved still further into the distal end of what was now recognized as a bronchus, disintegration of some of the central cells was noted The survival of a single peripheral layer which had compressed the surrounding mesenchyme resulted in the formation of a basement membrane and completed the formation of the epithelium-lined bronchial tube (fig 2 A)

The fact that in the rapidly growing fetal lung mitotic activity was most pronounced not at the tips of the elongating bronchi but in the undifferentiated mesenchyme peripheral to them has already been mentioned but deserves further emphasis If the elongation of bronchi resulted from the multiplication of cells of endodermal origin one would expect the tip of the growing bronchus to be the site of greatest mitotic activity as is the tip of a growing root of a plant

When glycogen first appeared, it was uniformly distributed in the cytoplasm of the activated mesenchymal cells As this chemical precursor of structural differentiation proceeded, the glycogen tended to accumulate in the peripheral end of differentiating epithelial cells but gradually shifted to the luminal or central ends of cells, where it persisted for a short time and was finally extruded into the lumen of the primitive bronchus Following the extrusion of the glycogen droplets, the cells became cuboidal, and their nuclei moved peripherally toward the basal ends of the cells that were now unmistakably epithelial in character The glycogen lying free in the lumen gradually disappeared, and by the third trimester of fetal life only small traces remained

#### ALVEOLAR DIFFERENTIATION IN UTERO

The process described in the previous paragraphs was the dominant feature of the development of the lungs during the first trimester of fetal life and led to the branching and elongation of bronchi which was preparatory to alveolar differentiation Not until the second and third trimesters of fetal development did alveolar differentiation become the dominant developmental change In contrast to the consolidation

and enlargement of mesenchymal cells which preceded bronchial differentiation, the development of the pulmonary alveolus was initiated by rarefaction. In preparation for alveolar development the mesenchymal cells at the tips of terminal bronchi became peripherally displaced as though by fluid. Clusters of cavities thus formed communicated with the terminal bronchial lumen. Subsequently the mesenchymal cells lining such cavities enlarged as glycogen appeared in their cytoplasm. With this enlargement they became cuboidal and took on the appearance of epithelium. There was considerable species variation in respect to the time at which the innermost mesenchymal cells that had been displaced to form these primitive alveoli acquired the appearance of epithelium. In the mouse there was a relatively long lag between the preliminary cavitation of the mesenchyma and the epithelial differentiation of the lining cells. In the guinea pig and in man epithelization followed closely after the appearance of the cavities.

The presence of a continuous epithelial lining of the immature alveoli was transitory, and about the time that alveolar formation was nearing completion the enlarged lining cells discharged their cytoplasmic accumulations of glycogen. As the glycogen was shed into the alveolar lumen, some of the lining cells disintegrated *in situ* (fig 7) and others desquamated (fig 8) as though they were being dislodged by the rapidly growing capillary loops which by this time were pushing up through and between them (figs 4, 5 and 6). Still others shrank and regressed to form the inconspicuous cells with small nuclei and scanty cytoplasm that have been variously referred to as septal cells (Lang<sup>15</sup>), epicytes (Clara<sup>16</sup>) or fixed macrophages (Fried<sup>2</sup>) and which are regarded by some as being of endodermal origin.

It would seem better to regard them as sensitive and plastic mesenchymal cells which are inactive and inconspicuous under normal conditions but which are capable of becoming active phagocytes when stimulated by the presence of foreign material and which may proliferate to form a continuous lining membrane in response to injury or may engage in more extravagant and atypical growth in such conditions as pulmonary adenomatosis or neoplasia.

#### DIFFERENTIATION OF FETAL LUNG TISSUE IN THE ANTERIOR OCULAR CHAMBER

Fetal lung tissues derived from guinea pigs, mice and rabbits were transferred to the anterior ocular chambers of adult guinea pigs by the technic<sup>9</sup> described by Dr H S N Greene, of New Haven, Conn.

15 Lang, F J J Infect Dis 37 430, 1925

16 Clara, M Ztschr f mikr-anat Forsch 40 147, 1936

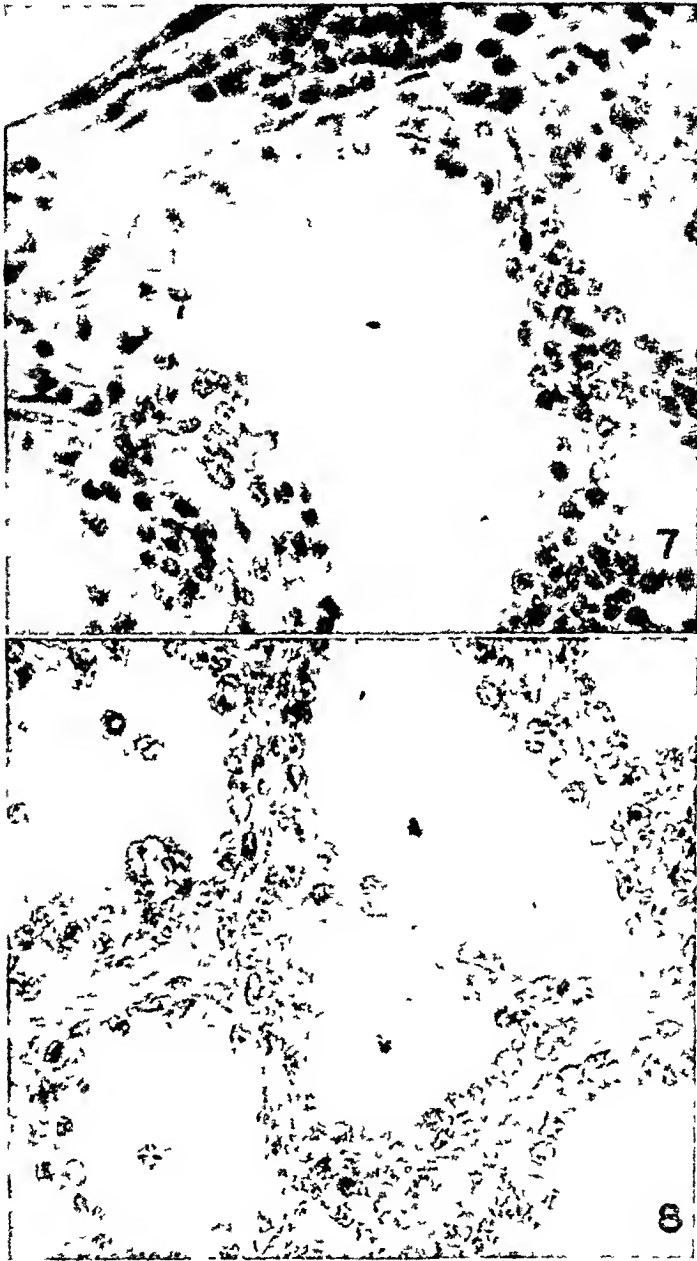


Fig 7—An alveolus of fetal guinea pig lung tissue that eight days earlier was transplanted from a 40 mm guinea pig embryo to the anterior chamber of an adult guinea pig's eye. On the upper left margin is the capsule of the transplant corresponding to pleura. Epithelial lining that is in the process of degenerating can be seen. The clear vacuoles contained glycogen, the extrusion of which accompanies the disintegration of some of the lining cells. Hematoxylin and eosin,  $\times 425$ .

Fig 8—Alveoli of the same 8 day old transplant shown in figure 7, in which can be seen the manner in which some lining cells become dislodged into the lumens as capillary development progresses. Notice the bare capillary separating two alveoli. Hematoxylin and eosin,  $\times 425$ .

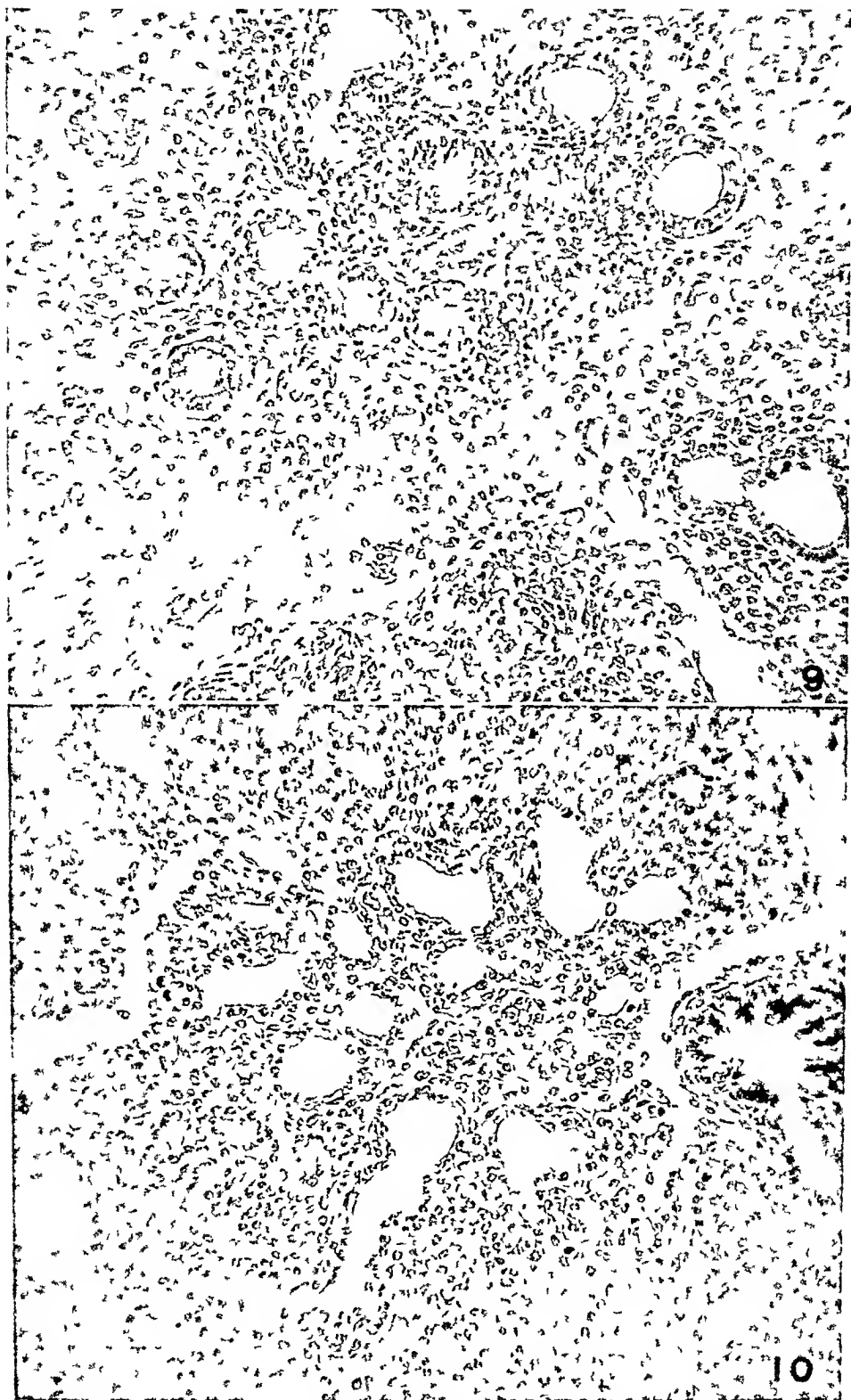


Fig 9—Lung of a 40 mm guinea pig embryo. At this stage most of the primitive lung is composed of an undifferentiated mesenchyme having an abundant amount of intercellular fluid except in the immediate vicinity of the differentiating bronchi, where the intercellular spaces are reduced and the cells more compactly arranged. Hematoxylin and eosin,  $\times 225$ .

Fig 10—Lung of a 54 mm guinea pig embryo showing the results of an additional ten days' differentiation in utero. The lung is now comprised principally of small bronchi lined by cuboidal cells and separated from one another by compact mesenchyme. The loosely arranged mesenchyme composed of spindle cells seen in the 40 mm fetus has almost completely disappeared. Hematoxylin and eosin,  $\times 225$ .

Dr Greene contributed assistance and advice in the development of this phase of the investigation. I had not tried this particular experimental technic before undertaking the investigation herewith reported, and many of the initial attempts to secure heterotopic growth of lung tissue were unsuccessful. With increasing experience it was found that the majority of such attempts were successful in that the transplanted lung tissue remained viable, underwent a transplantation phase of growth and differentiation and remained free of infection.

Survival and growth of transplants did not appear to be influenced by the strain, age or sex of the host animals. A fetus to be used as a source of lung tissue was removed by hysterotomy, with the animal under "pentothal sodium" anesthesia. When the uterus contained more than one fetus, one was taken for transplantation experiments and the others were left to develop in utero so as to serve as litter mate controls. Usually the controls were removed at the time that the transplants were excised for microscopic study. Although hysterotomy was frequently followed by abortion when performed during the terminal period of pregnancy, it rarely caused abortion when performed during the first trimester.

The observations reported in the succeeding paragraphs are derived from 38 transplants of lung tissue from guinea pig fetuses having crown-rump measurements ranging between 24 and 95 mm. The usual procedure was to divide the lung of the donor fetus into between four and six fragments of approximately similar size and shape. One of these was immediately fixed for control study and 1 to 2 mm fragments of the others were transplanted to anterior ocular chambers. The transplants were removed for microscopic study after periods of heterotopic survival ranging between four and ninety days.

In general the incidence of successful transplants bore an inverse relationship to the age of the donating fetus. In experienced hands the complete failure of a transplant derived from a fetus during the first or second trimester of gestational development to survive was unusual. The majority of such transplants not only survived but underwent a period of active growth, usually filling the anterior chamber. Although the majority of transplants from older fetuses remained viable and usually survived for weeks before atrophy could be perceived by clinical examination, active growth of such tissue was the exception rather than the rule.

After being introduced into the anterior chamber the transplanted fragment usually remained relatively unchanged for a period of between twelve and thirty-six hours. By the end of thirty-six hours the transplant was characteristically pink and visibly vascularized. New blood

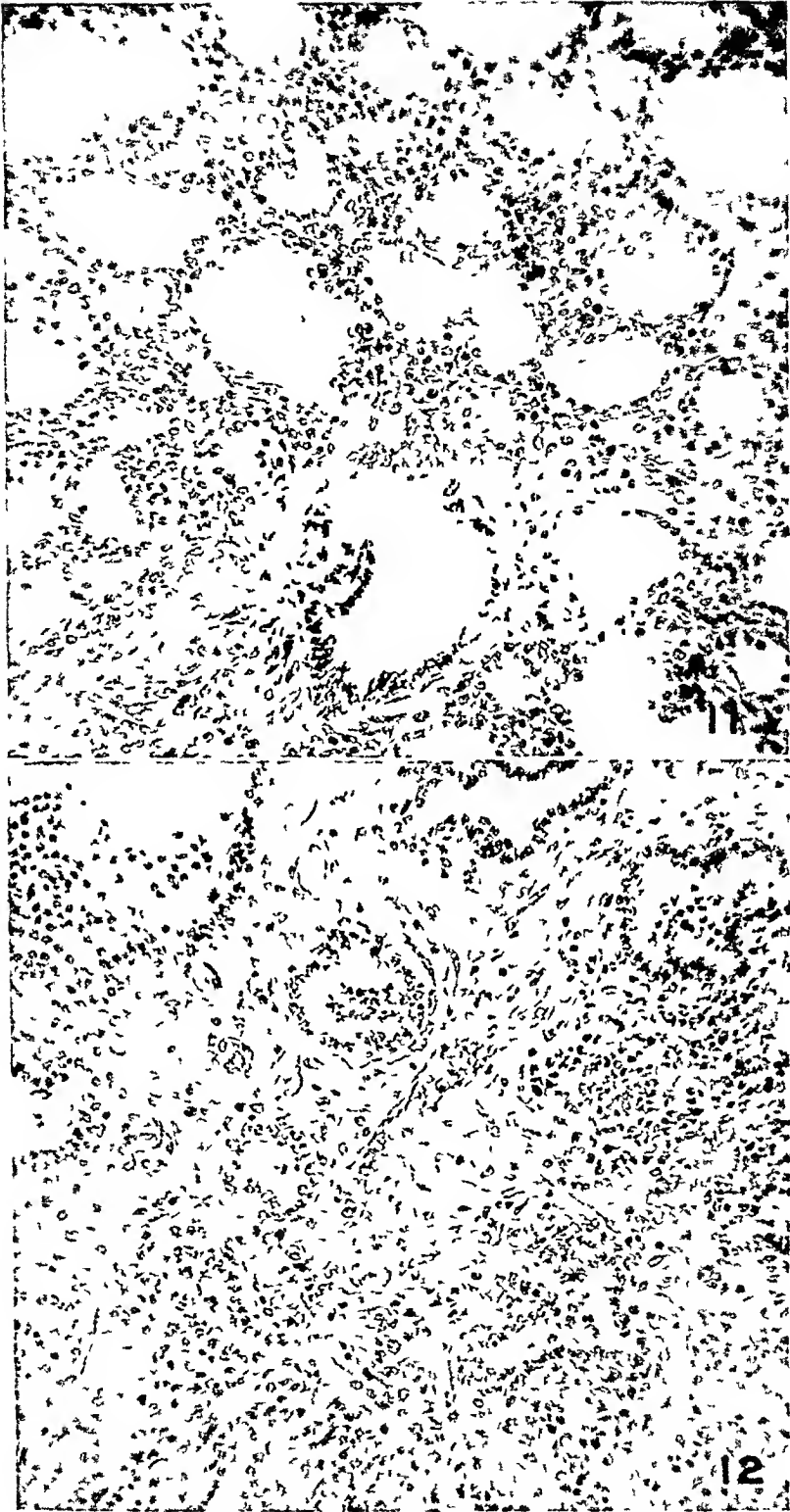


Fig 11—Eight day old transplant of fetal guinea pig lung. This tissue was transplanted from a 40 mm embryo to the anterior chamber of an adult guinea pig's eye. The differentiation of this transplant has been greatly accelerated, and the degree of differentiation shown would not have been attained short of near-gestational maturity (about seventy-two days) in utero. Well developed alveolar structure and bronchi are shown. Most of the alveoli are lined by low cuboidal epithelium, but in some bare capillaries can be seen. Hematoxylin and eosin,  $\times 225$

vessels extending between the iris or the cornea and the transplant were frequently visible by the end of the third day

*Behavior of Fetal Lung Tissue Transplanted in the Anterior Chamber of an Adult Guinea Pig's Eye*—Microscopic examination of fetal lung tissue after varying periods of heterotopic growth disclosed a sequence of four more or less well defined phases

During the initial post-transplantation phase, which usually lasted for twelve to thirty-six hours, the transplant showed little or no change. A mild inflammatory reaction consisting of edema and hyperemia was elicited in the adjacent host tissue. Although failure to acquire a blood supply during this period usually resulted in degeneration and necrosis, an occasional transplant remained dormant for many days before growing or showing evidence of having acquired a blood supply from the host.

If, as was usually the case, the transplant acquired an adequate blood supply during the first few days, active proliferation occurred and continued for days or weeks. This proliferation took any one of several different forms. In some instances growth and differentiation took place in an orderly fashion and, although it often occurred either more or less rapidly than would have been the case if it had been taking place in the intact fetus, resulted in well differentiated alveoli and air passages with little or no residuum of undifferentiated mesoderm (fig 11)

In other instances the proliferative phase was represented by multiplication of the mesoderm of the primitive blastema with little or no evidence of differentiation (fig 13). The latter type of growth resulted in a cellular mass of tissue sometimes having a microscopic appearance remarkably similar to that of a rapidly growing spindle cell tumor. In transplants of this type there appeared to be actual dedifferentiation of many partially differentiated cells to a more primitive form. In some transplants only the most highly differentiated elements, such as the cartilage and the epithelium of the larger bronchi, remained to distinguish the original character of the tissue (fig 12). In still others proliferation proceeded nonuniformly and atypically, leading to relatively normal organoid differentiation in some places and to an over-

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Fig 12—Eleven day old transplant of fetal guinea pig lung. This tissue was transferred from a 40 mm embryo to the anterior chamber of an eye of an adult guinea pig. The interstitial tissue that normally appears as in figures 1 and 2 has largely disappeared, being replaced by an edematous, loose fibrillar connective tissue. The remaining cells are disintegrating. The cytoplasm stains poorly. The nuclei are pyknotic. In some areas the spindle-shaped cells indicate that local tissue is being transformed to fibrous tissue. The more differentiated bronchial epithelium shows less evidence of degeneration. There is no reason to believe that this transplanted fetal tissue ever differentiated as did that shown in figure 11. Hematoxylin and eosin,  $\times 225$



growth of the mesoderm of the primitive pulmonary blastema in others. Between these two extremes, all manner of abnormal or unbalanced growth took place.

After the initial period of active proliferation the transplant entered a static phase in which there was neither growth nor perceptible retrogression. This often lasted for days or weeks before passing on into the next and final phase of the life of the transplant.

The last phase in the sequence was one of involutional fibrosis and atrophy. The cicatrix, which was at first loosely cellular and edematous (figs 12 and 14), eventually became dense and contracted.

The behavior of the individual components of such transplants was often exceedingly complex. It was difficult or impossible to draw any inferences as to the effect which the heterotopic environment had on pulmonary differentiation from a study of a single or, for that matter, of several individual transplants. It was only through a study of many transplants of varying age and by comparing one with another as well as with the control tissues that certain behavior patterns were recognized.

In the majority of transplants that had resided in anterior chambers for more than a week, parts of the original tissue had either remained static, with no apparent further differentiation, or had been overgrown by primitive connective tissue. Interspersed throughout the mesoderm were bronchi in varying stages of development. The quantitative relationships between bronchi and mesoderm usually varied according to the gestational age of the fetus from which the transplant was derived, the length of time that had elapsed since the transplantation and the extent to which the particular portion of the transplant being examined had secured an adequate blood supply from adjacent host tissue. Bronchi examined by means of serial sections were found to be blind tubes or cysts (fig 20). Some were static, and others showed evidence of terminal branching and growth similar to that observed in fetal lung tissue developing *in situ*. Studies of serial sections stained for glycogen indicated that terminal growth and differentiation of bronchi in heterotopic lung tissue represented first a chemical and later a structural differentiation of the mesoderm occurring in a manner similar to that observed *in utero*.

The development of most of some and parts of many of the transplants went on to the formation of the alveolar systems associated with terminal bronchi. Occasionally such differentiation was so balanced and complete that it was impossible to tell from a single field examined under high magnification whether the differentiation of the tissue being examined had occurred in an intact fetus or in an ocular transplant.

of fetal lung. Studies of alveolar differentiation by means of serial sections stained for glycogen revealed that in transplants the process was similar to, or identical with, that which occurs in the intact fetus. The

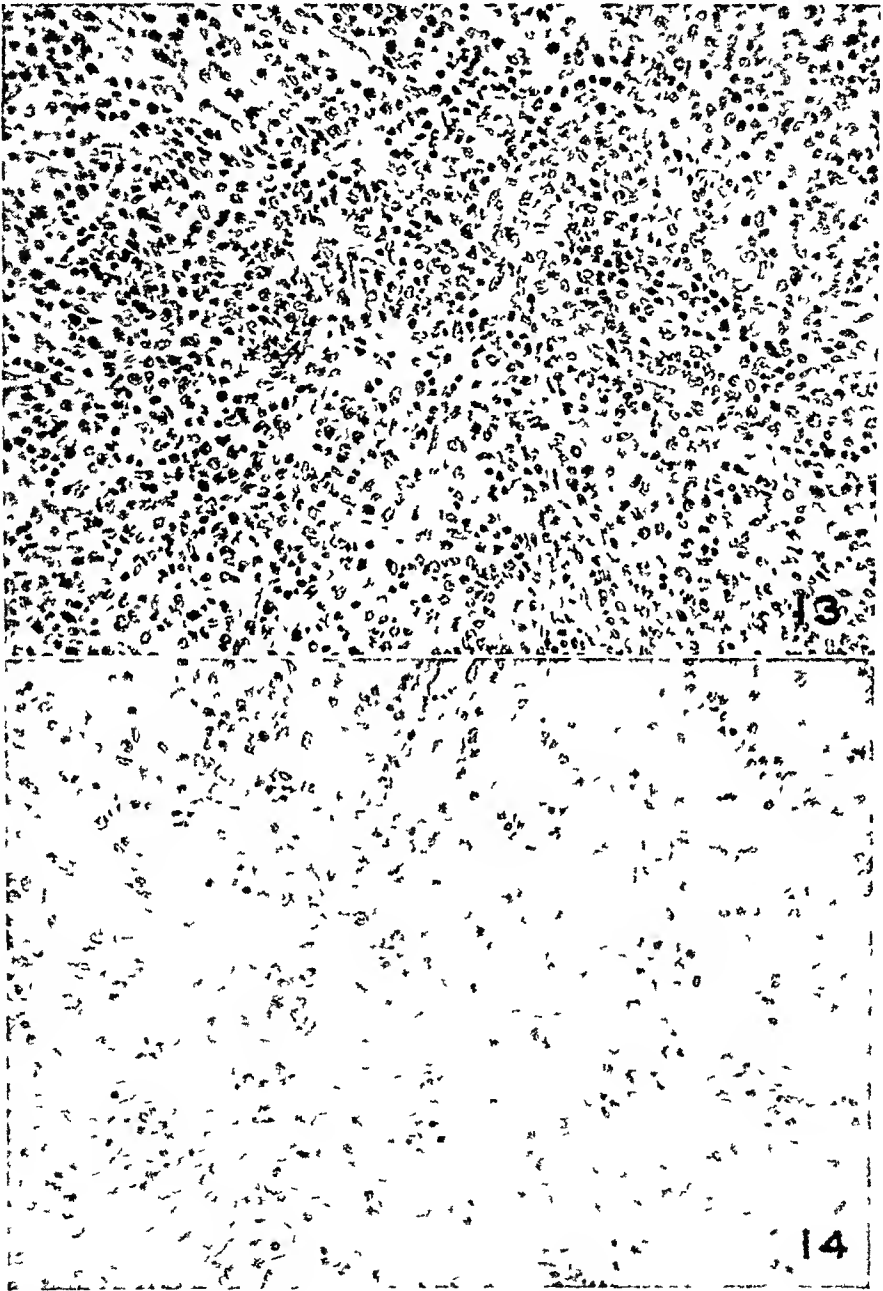


Fig 13—Twenty-five day old transplant of fetal guinea pig lung. This tissue was transferred from a 40 mm embryo to the anterior chamber of an adult guinea pig's eye. Most of the transplant is made up of undifferentiated mesoderm in which there are only occasional abortive attempts at bronchial formation. Hematoxylin and eosin,  $\times 225$ .

Fig 14—Thirty-two day old transplant of fetal guinea pig lung. This tissue was transplanted from a 40 mm embryo to the anterior chamber of an eye of an adult guinea pig. The relatively acellular, edematous fibrous tissue is near the end stage of the regressive changes seen in figure 12. Hematoxylin and eosin,  $\times 225$ .

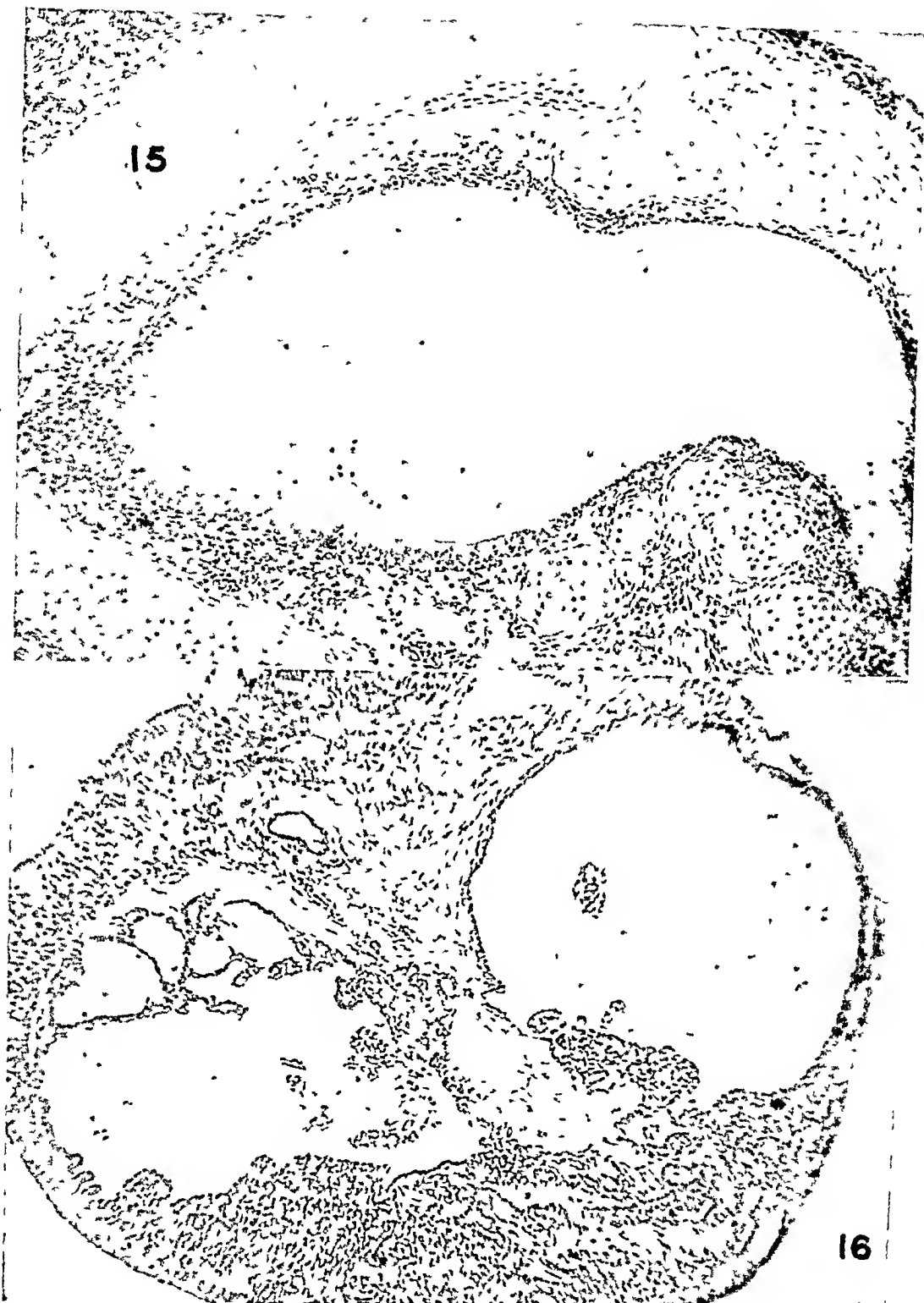


Fig 15—A greatly dilated bronchus developing in an 8 day old subcutaneous transplant of fetal mouse lung. This tissue was transplanted from a 14 mm mouse embryo to an adult mouse. The cartilage is well differentiated. No recognizable smooth muscle is present. The epithelial lining is made up of ciliated columnar epithelial cells in one area but gradually gives way to cuboidal and squamous epithelial cells in other areas about the bronchus (see figs 17, 18 and 19). Hematoxylin and eosin,  $\times 100$ .

Fig 16—Cysts developing in a subcutaneous transplant of fetal mouse lung. This tissue was transferred from a 15 mm embryo to an adult mouse and was removed after ten days. Abortive alveolar formation can be seen on the left. These cysts are lined by squamous epithelium which in some areas blends imperceptibly with the surrounding fibrous stroma. Hematoxylin and eosin,  $\times 65$ .

assertion that in utero the expansion of pulmonary alveoli is related to the patency of bronchial commissures and the aspiration of amniotic fluid was contradicted by the fact that expanded alveoli containing only occasional flattened lining cells were observed in ocular transplants (figs 8 and 11). Similarly the dilated and even cystic bronchi observed in transplants (figs 15, 16 and 20) indicated that such alterations are not necessarily predisposed to by extrapulmonary stresses.

The type of bronchial epithelium encountered in the transplants was exceedingly variable. Thus in a single cyst ciliated epithelium was seen to merge gradually into a cuboidal or a stratified layer (figs 15, 17, 18 and 19). In some instances the variation of the bronchial epithelium appeared to be related to the manner in which the transplant had secured its new blood supply. Normal differentiation tended to occur where an adequate blood supply was obtained soon after transplantation, and abnormal or atypical differentiation was most pronounced where vascularization was poorest. Thus, the cells lining bronchi located in the well vascularized peripheral zone of a transplant usually differentiated in a normal manner, while in areas farther removed from the periphery the epithelium was more commonly of the squamous type. These differences appeared to be due to altered differentiation rather than to metaplasia.

#### DIFFERENTIATION OF FETAL LUNG TISSUE IN SUBCUTANEOUS TRANSPLANTS

The subcutaneous transplantation site was explored briefly and found to differ in no essential way from the intraocular site as regards ease of growth and differentiation of fetal tissue. The use of mice facilitates the use of larger numbers of animals. The growth of the transplant cannot be visualized, of course. Cyst formation, bronchiectasis and bizarre epithelial overgrowths occurred somewhat more frequently in the subcutaneous transplants than in the intraocular group. Subcutaneous transplants evoked little or no fibrous reaction in mice, whereas an overwhelming proliferation of fibrous tissue occurred about such transplants in guinea pigs. The subcutaneous transplants never attained that degree of differentiation seen in comparable intraocular transplants.

#### COMMENT

The implications of the foregoing observations are many. They tend to unify and offer a rational explanation for several divergent views concerning pulmonary embryology and pathology, which in the past have occasioned considerable speculation and wide difference of opinion.



Fig 17—Normally differentiated ciliated columnar epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin,  $\times 900$ .

Fig 18—Stratified cuboidal epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin,  $\times 900$ .

The concept of the specificity of germ layers is so firmly implanted in the medical literature of the past one hundred years that it is probably a fact that many have never had occasion to doubt its validity. There is, however, a considerable body of evidence contradicting the doctrine of absolute specificity of the germ layers as enunciated in the last century. Oppenheimer<sup>17</sup> reviewed the subject in 1940 and cited many notable exceptions. In conclusion she stressed such factors as the special metabolism of certain tissues of the developing embryo, the topographic position of the cells and the interactions of various cells, one with another, as being of vital importance in controlling their differentiation.

The results of this study detract from the concept that the ability of a cell to differentiate is necessarily dependent on the germ layer from which it was derived. It would appear that the entire lung is derived from totipotent tissue whose direction of growth and differentiation is induced by the stimulus rather than by the proliferation and migration of previously formed structures.

However radical this concept of bronchial epithelium derived by differentiation from mesoderm may seem there is evidence from other sources to support it. Hunt<sup>18</sup> has shown that in the chick after removal of the presumptive endoderm the mesoderm can form gut and its derivatives. Papanicolaou<sup>18</sup> observed the differentiation by which undifferentiated mesodermal cells became uterine epithelium in the tunica propria of the guinea pig uterus. The prevalent but erroneous idea that mesoderm does not give rise to epithelial structures need cause no difficulty, for there are the uterus and kidney to illustrate this phenomenon.

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17 Oppenheimer, J. M. *Quart. Rev. Biol.* **15** 1, 1940.

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Fig. 19—Stratified squamous epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin,  $\times 900$ .

Fig. 20—A dilated bronchus in tissue transplanted eight days earlier from a lung of a 44 mm guinea pig embryo to the anterior chamber of an adult guinea pig's eye. Note that the well differentiated epithelium seen in some areas is continuous in other areas with epithelium which has not yet differentiated from the surrounding mesoderm. Along the lower cyst wall one may observe the manner in which the formation of a bronchus can precede the differentiation by which adjacent mesoderm becomes epithelium, an outpocketing has produced a tube which is as yet nonepithelialized. Hematoxylin and eosin,  $\times 225$ .

Fig. 21—Longitudinal section of a bronchus developing in a 6 day old subcutaneous transplant of fetal mouse lung. This tissue was moved from a 14 mm embryo to an adult mouse. Note that the epithelium is continuous with that of the larger bronchus above and that toward the tip of the bronchus it gradually fuses with the surrounding mesoderm. The gradual alteration by which undifferentiated mesodermal cells become epithelium can be seen along the bronchial wall. Hematoxylin and eosin,  $\times 425$ .

From the point of view of the pathologist the observation of the mesodermal origin of the lung serves to unify many observations which have been incompletely understood. A common genetic origin of the many types of pulmonary neoplasms has been postulated by several observers. The origin of most true pulmonary cancers has been traced to the bronchial epithelium on purely morphologic evidence. However, the marked pleomorphism and the frequent occurrence of sarcomatous elements have been difficult to explain if one considers the bronchial epithelium to be of endodermal origin and this germ layer specific for epithelial structures. The fact that bronchial epithelium can and regularly does differentiate from mesoderm coincides remarkably with its derivatives which appear in neoplasms of the lung.

The difficulty of morphologic classification of cancers of the lung is readily explained and simplified by the consideration of the mesodermal origin of the structures from which they arise. The potencies of bronchial epithelium are those of mesoderm. The sarcomatous changes observed in transplanted pulmonary carcinomas by Stewart, Grady and Andervont<sup>20</sup> and others<sup>21</sup> become more readily explainable. Although these authors draw no definite conclusions from their observations, they were forced to consider the possibility that pulmonary carcinomas were derived at least in part from mesoderm.

Again when one considers the noncancerous bronchial tumors the frequent observation of neoplastic cartilaginous and osseous tissue is not surprising. The necessity of classifying them as mixed tumors as Womack and Graham<sup>22</sup> have done would be abolished.

Finally the bronchiectasis observed in many transplants may have a bearing on the etiology of this disease, especially in those cases in which it is associated with other developmental anomalies. For example, in transposition of the viscera it is possible that the failure of the pulmonary veins to establish connection with the heart and the consequent necessity of persistence of the bronchial veins produce a temporary nutritional defect somewhat similar to that in transplants.

The relatively large cysts that occasionally occur in the transplants, almost replacing them, would seem to be closely analogous to congenital cysts of the human lung.

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18 Hunt, T. E. *Anat Rec* **68** 349, 1937

19 Papanicolaou, G. N. *Am J Obst & Gynec* **25** 30, 1933

20 Stewart, H. L., Grady, H. G., and Andervont, H. B. *J Nat Cancer Inst* **7** 207, 1947

21 Wells, H. G., Slye, M., and Holmes, N. F. *Cancer Research* **1** 259, 1941  
Breedis, C., Robertson, T., Osenkop, R. S., and Furth, J. *ibid* **2** 116, 1942

22 Womack, N. A., and Graham, E. A. *Arch Path* **26** 165, 1938

## SUMMARY

The genesis of mammalian pulmonary tissue as observed in the fetus in utero and in transplants in anterior ocular chambers and subcutaneous sites in homologous adults is described. Evidence that the cells which line the bronchi and the alveoli originate from mesoderm is presented, and the nature of the lining of fetal and adult pulmonary alveoli is described. The intracytoplasmic accumulation of glycogen observed in the developing lung has been correlated with the differentiation of bronchi and alveoli. Differences induced in organization and in cytologic differentiation by altering the environment and sources of nutrition of fetal lung tissue are described. Attention is called to the similarity between certain changes induced in fetal lung tissue by transplantation and those encountered in adult lung tissue presumably as a result of congenital dysplasia.



# LESIONS INDUCED IN RABBITS BY CHOLESTEROL FEEDING, WITH SPECIAL REFERENCE TO THEIR ORIGIN

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**T**HAT atherosclerosis and xanthomatosis occur in rabbits fed a high cholesterol diet has been reported by many investigators. The earlier literature bearing on experimental atherosclerosis has been reviewed by Anitschkow<sup>1</sup>. The results of studies bearing on certain aspects of the problem have been summarized by various authors, including Weinhouse and Hirsch<sup>2</sup> and Leary<sup>3</sup>. The literature related to xanthomatosis has been summarized particularly by Thannhauser<sup>4</sup> and by Galloway, Broders and Gharmley<sup>5</sup>.

The most prominent cellular components of both the atherosclerotic plaque and xanthoma in the early phases of their development are the so-called foam cells. Nearly all the investigators who have studied these cells have reported data supporting the theory that they are derived from the reticuloendothelial system. According to the point of view advanced by Leary<sup>3</sup> in 1941, the foam cells of the atherosclerotic lesions represent lipophages which invade the intima from the blood stream. Gordon<sup>6</sup> has attempted to explain such invasion on the basis of certain data regarding the behavior of the cellular elements of the circulating blood and theoretic considerations. Contrary to this point of view, Moreton<sup>7</sup> has pointed out that when lipids are present in the blood the nutrient lymph which enters the intima from the blood

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1 Anitschkow, N, in Cowdry, E V Arteriosclerosis, New York, The Macmillan Company, 1933, p 271

2 Weinhouse, S, and Hirsch, E F Arch Path **30** 856, 1940

3 Leary, T Arch Path **32** 507, 1941, **37** 16, 1944

4 Thannhauser, S J Lipidosis Diseases of the Cellular Lipid Metabolism, New York, Oxford University Press, 1940

5 Galloway, J D B, Broders, A C, and Gharmley, R K Arch Surg **40** 485, 1940

6 Gordon, I Arch Path **44** 247, 1947

7 Moreton, J R Science **107** 371, 1948

plasma carries large lipid particles, which become lodged there. These lipid particles, according to his point of view, incite a local foreign body reaction which results in their being ingested, with formation of typical foam cells. This he regards as the characteristic histologic feature of the genesis and the development of atherosclerotic lesions.

The present investigation was undertaken to obtain additional data relative to atherosclerosis and xanthomatosis in rabbits fed a high cholesterol diet. Particular attention has been given to the cholesterol content of the blood, the origin of the foam cells and the sequence of changes in atherosclerotic lesions following their initiation in both large and small arteries and in the associated lesions of viscera and of the autonomic ganglions and nerves. An attempt has been made also to correlate the histopathologic changes of the autonomic ganglions and nerves with the atherosclerotic and other visceral lesions which are associated with the induced hypercholesteremia.

#### METHODS

The rabbits used were young adults when the experimental feeding was initiated. The basic diet consisted of a commercial rabbit chow<sup>8</sup>. Twenty animals received, in addition to the basic diet, 1 Gm of cholesterol in 25 Gm of dehydrogenated vegetable oil daily. Several were fed less than 1 Gm of cholesterol daily. The diet was prepared by dissolving the cholesterol in melted dehydrogenated vegetable oil and mixing this with chow pellets. The oil, including the cholesterol, became adherent to the chow pellets. In general, all the food given to the individual animal was eaten.

Several of the animals fed the high cholesterol diet were killed 30 to 40 days after the initiation of the diet. Others were killed at graded intervals up to 380 days. Before an animal was killed, its physical condition was noted, and an examination was made to determine the presence or the absence of recognizable xanthoma. In some instances blood pressures were determined. Immediately after the death of the animal, blood was drawn directly from the heart for the determination of its cholesterol content. The tissues desired for study were removed, fixed and prepared by various histologic technics.

#### BLOOD CHOLESTEROL LEVEL

The rabbits fed the high cholesterol diet became hypercholesteremic relatively early. Determinations carried out on one animal which had received 1 Gm of cholesterol daily for 46 days and another which had received the same amount daily for 62 days indicated a blood cholesterol level of approximately 3,550 mg

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<sup>8</sup> "Purina rabbit chow checkers" (complete ration) was used, made by the Ralston Purina Company, St. Louis. The company has described the chow as follows: The ingredients are wheat germ, soybean oil meal, corn germ meal, alfalfa leaf meal, wheat middlings, ground oats, cornmeal, blackstrap molasses, calcium carbonate and iodized salt. Chemical analysis shows protein 16.45, fat 4.06, fiber 7.93, ash 3.75 and nitrogen-free extract 52.60 per cent. Mineral analysis shows calcium 0.821, phosphorus 0.36, potassium 0.753, sodium 0.317, magnesium 0.150, sulfur 0.222 and chlorine 0.386 per cent.

per hundred cubic centimeters in both animals. Determinations carried out on control rabbits indicated a range in the blood cholesterol level of 137 to 243 mg per hundred cubic centimeters. Rabbits which were fed 0.5 Gm of cholesterol or less daily exhibited relatively small elevations of the blood cholesterol levels. One animal fed 0.2 Gm daily for 200 days showed a level of 1,320 mg per hundred cubic centimeters. Another fed 0.4 Gm daily for 87 days showed a level of 1,770 mg. Determinations carried out on rabbits which had been fed 1 Gm of cholesterol daily for 293 to 380 days indicated a range in the blood cholesterol levels of 2,400 to 2,750 mg per hundred cubic centimeters.

#### ATHEROSCLEROSIS

*Nature and Distribution of Lesions*—The earliest lesions have been observed in the thoracic aorta. Our data indicate considerable individual variation with respect to the development of atherosclerotic lesions in rabbits fed the same diet. The feeding of less than 1 Gm of cholesterol daily, furthermore, is less effective than the feeding of 1 Gm daily. One animal killed 46 days after the initiation of a diet including 1 Gm of cholesterol daily had early lesions in the aorta and in some other elastic arteries and initial lesions in some of the smaller muscular arteries. The blood cholesterol determination indicated a level in excess of 3,500 mg per hundred cubic centimeters in this animal. Another rabbit which had been fed 0.4 Gm of cholesterol daily for 87 days showed only early atherosclerotic lesions, although its blood cholesterol determination indicated a level of 1,770 mg per hundred cubic centimeters. Rabbits which had been fed 1 Gm of cholesterol daily for 150 to 200 days had well developed intimal atherosclerotic plaques in the aorta and other large arteries and intimal lesions in many of the small arteries, although most of the smaller arteries, as observed in sections of the viscera, exhibited no recognizable lesions.

In the aorta the maximal thickness of fully developed intimal plaques exceeded twice the thickness of the normal vessel wall. In some instances the plaque extended entirely around the lumen but was not of uniform thickness. Animals having large intimal plaques in the aorta also had large intimal plaques in the common carotid, the coronary and other large arteries, which in most sections did not extend entirely around the lumen. Some relatively small branches of the coronary arteries were partially occluded by intimal thickenings. In other viscera some arteries, including prearteriolar branches, exhibited initial atherosclerotic lesions, but most of the muscular arteries, as observed in sections of the organs, revealed no apparent lesions.

In a rabbit fed the high cholesterol diet 293 days, the blood cholesterol determination of which indicated a level of 2,400 mg per hundred cubic centimeters, the intimal plaques in the aorta were no larger than those observed in the aortas of rabbits which had been fed cholesterol for only 200 days, but they differed from the latter in their composition. The most striking differences were a marked increase in the amount of the collagenous tissue and a marked decrease in the number of foam cells present. Most of the remaining foam cells exhibited shrinkage of both the cytoplasm and the nucleus. Sections of the aorta of this rabbit revealed extensive lesions of the media, particularly in areas in which the intimal plaques were most extensively developed. In some areas destruction of the media was almost complete (fig 2).

Sections of the common carotid arteries of this animal exhibited intimal plaques comparable in size to those observed in the carotid arteries of rabbits fed cholesterol only 200 days. These plaques also showed a proportionate increase of the amounts

of collagenous tissue present (fig 1) Sections of the coronary arteries exhibited lesions which were comparable to those in the common carotid arteries

The percentage of the arteries in the viscera which presented atherosclerotic lesions, as observed in sections of the organs, was markedly greater than that noted in the animals which had been fed cholesterol only 200 days or less Occasional small arteries observed in sections of various organs, including the heart, the intestine and the kidney, were completely or almost completely occluded Most of the very small arteries and the arterioles exhibited apparent thickening of the walls and constriction in some degree

In rabbits which had been fed cholesterol for 300 to 380 days the atherosclerotic lesions were not appreciably more extensive than in the rabbits fed cholesterol for approximately 300 days The damage of the media of the arteries was somewhat more extensive The proportionate amounts of collagenous material in the lesions also was greater In many instances sections revealed definite stratification due to alternating layers characterized respectively by preponderance of fibrous tissue and foam cells Some of these lesions showed, in contact with the inner elastic membrane, a relatively thick layer, which consisted almost exclusively of foam cells and intercellular lipid material (fig 2)

Initial atherosclerotic plaques consisted mainly of foam cells and intercellular lipids located between the endothelium and the internal elastic membrane (fig 3) Fibrous tissue including cellular elements of connective tissue origin became apparent early and were constantly present in the later lesions

Sections of the aortas of cholesterol-fed rabbits stained with sudan black exhibited lipid material in larger amounts In the atherosclerotic lesions lipids were present both within the cells and between them The intracellular lipids appeared mainly in the forms of granules and globules of varying sizes In early lesions the foam cells were heavily laden with lipids In the more advanced lesions most of the foam cells contained but little stainable lipid material In the media the lipids appeared mainly in granules and globules of varying sizes lying between the elastic fibers Sections of the aortas of control rabbits, stained in the same manner, contained only traces of lipids except in the adventitia

Sections of arteries of cholesterol-fed rabbits stained with Nile blue sulfate, which is specific for unsaturated fatty acids, exhibited these substances in large amounts, whereas sections of the same arteries of control rabbits, stained in the same manner, exhibited only traces of unsaturated fatty acids In general, the distribution of the unsaturated fatty acids paralleled that of the lipids which reacted positively to sudan black The material which reacted positively to the Nile blue sulfate probably represents triolein derived from the dehydrogenated vegetable oil in which the cholesterol had been dissolved in the preparation of the diet

Sections of arteries of cholesterol-fed rabbits subjected to the Romieu test<sup>8a</sup> for cholesterol exhibited large amounts of this material in the atherosclerotic plaques, both in the foam cells and in the ground substance, but none in the media or in the adventitia

*Development of Lesions*—The initial atherosclerotic lesion appears as a slight thickening of the intima The area of the lesion may include a few foam cells located between the endothelium and the inner elastic membrane In some instances the endothelial cells in the area of the lesion appear to be thickened and to contain lipid material in their cytoplasm They are also more numerous than in unaltered areas This suggests endothelial cell proliferation In some instances there were observed just beneath the endothelium, cells which, with respect to size, form and

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8a Romieu M Compt rend Acad d sc 184 1206, 1927

lipid content, appeared to represent intermediate stages of a transformation of endothelial cells into foam cells. During their early phases many of the plaques consist essentially of aggregates of foam cells in the intima, which are covered with endothelium. An individual lesion usually involves a circumscribed area the greatest dimension of which is parallel to the axis of the vessel. It is narrow at first but may increase in width until it extends completely around the lumen of the vessel. The tumor, which has been designated the intimal plaque, is of variable thickness. As it increases in size, fibrous tissue becomes apparent within it. The earliest connective tissue cells and fibers appear between groups of foam cells. As the fibers become more abundant, the groups of foam cells become more widely separated. In the denser parts of the fibrous tissue foam cells in small groups or individually appear to be embedded in a fibrous matrix. These cells are smaller than the typical foam cells, and their nuclei are shrunken. They have lost part of their lipid content. In the larger masses, particularly in those close to the endothelium, the foam cells remain unaltered. The fibrous material in the plaque, as determined by differential staining methods, is mainly collagen. Fibroblasts appear mainly between the collagenous fibers. In rabbits which had been fed cholesterol 300 days or longer, many of the intimal plaques consisted mainly of connective tissue, with relatively small masses of foam cells embedded in it.

In the area in which the plaque is thickest, in most instances some tissue elements of the intima and the inner layers of the media appear necrotic. As collagenous fibers develop in the intimal plaque, such fibers arise also in the portion of the media in which necrosis has taken place. In many instances the media was materially reduced in thickness in the area covered by an intimal plaque, owing to destruction of both muscular and elastic elements. In some instances the media was almost completely destroyed in a limited area (fig 2). Not infrequently, small groups of foam cells were observed in the media in areas in which the muscular and the elastic elements had undergone necrosis. Calcium deposits occurred in greatest abundance in these areas. In certain instances groups of foam cells appeared also in the adventitia overlying the lesion of the media. Lesions of this kind which involve the entire thickness of the arterial wall undoubtedly constitute areas of profound weakness.

The atherosclerotic plaques of the small muscular arteries, as observed in sections of viscera, in most instances did not extend entirely around the lumen but consisted of one or more groups of foam cells and some collagenous fibers (fig 4). In many sections the endothelium appeared to be intact. Even a minute lesion causes partial occlusion of the lumen. In many instances occlusion of the lumen was complete. Reduced thickness of the media was not uncommon.

In the smallest arteries complete occlusion was not uncommon even though the lesions were not extensive and had not yet reached the fibrotic stage. In an arteriole a group of no more than 3 or 4 foam cells may cause complete occlusion.

*Genesis of Foam Cells*—Our observations relative to the origin of the so-called foam cells which make up the major portion of the intimal plaque in the early phases of its evolution support the theory that they arise locally and are derived mainly from the endothelium. In sections of the aorta and other large arteries which exhibit early lesions the endothelium in the area of the lesion, in many instances, was thicker than the unmodified endothelium, and the endothelial nuclei were closer together. The endothelial cells apparently had increased in number, suggesting endothelial cell proliferation. Some of the thickened endothelial cells were so laden with lipid material that their cytoplasm presented a vacuolated appearance resembling that of the cytoplasm of the foam cells beneath the endo-

thelium Mitotic division of endothelial cells has not been observed and probably does not occur in the developing atherosclerotic lesion In some thickened endothelial cells the nucleus appeared to be constricted in a plane at right angles to its long axis Binucleate endothelial cells also have been observed Dumbbell figures

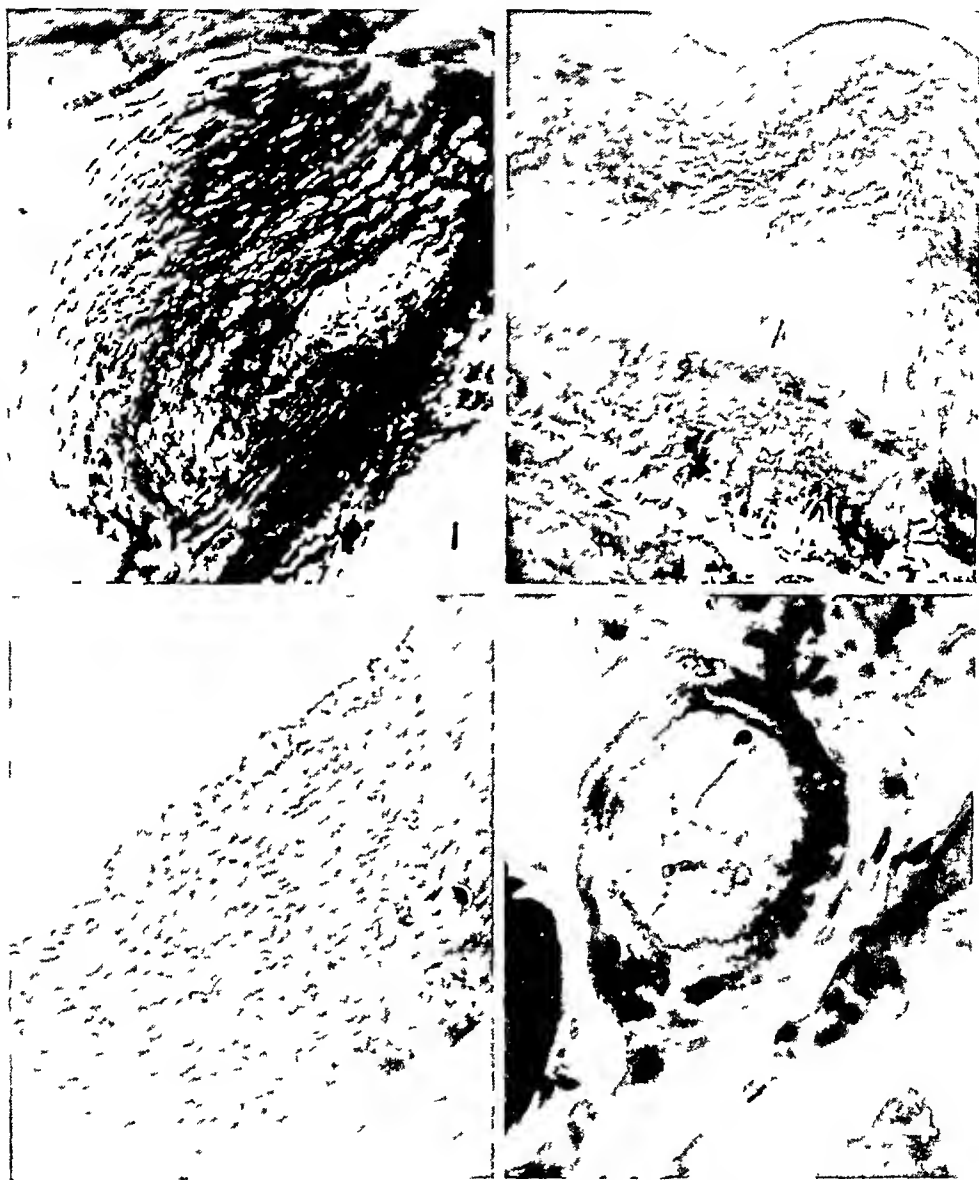


Fig 1—Section of an atheromatous carotid artery showing an intimal plaque

Fig 2—Section of an atheromatous aorta with stratification of an atheroma and partial destruction of the media

Fig 3—Section of an aorta showing an early atheromatous lesion

Fig 4—Section of a small atheromatous artery of the wall of the intestine, partially occluded by foam cells

such as are characteristic of nuclei undergoing amitotic division have not been observed in the endothelium Such figures have been observed frequently just beneath the endothelium and in the deeper portions of intimal plaques In intimal plaques which were undergoing rapid development, cells which appeared to represent

intermediate stages, with respect to form and lipid content, between the thickened endothelial cells and the fully differentiated foam cells were not uncommon just beneath the endothelium

Thickening of endothelial cells and assumption of spheroid forms as the cytoplasm becomes laden with lipid material have been observed frequently in small arteries. Such cells protrude into the lumen, sometimes singly and sometimes in small groups. In sections of the smallest arteries the foam cells retained their positions in the endothelium. In sections of arteries which were large enough to permit the development of intimal plaques comprising many foam cells, the latter usually appeared to be covered by endothelium.

Early atherosclerotic lesions appear to be limited to the intima. If all the foam cells arise locally, those in the lesions of the smaller arteries must be derived solely from the endothelium, for in these vessels the intima includes no other cellular elements. In the larger arteries the intima includes some cells of connective tissue origin, some of which undoubtedly have the capacity to become laden with lipids and to differentiate into foam cells. Even in these vessels the endothelium apparently constitutes the chief source of the foam cells.

In order to test the theory that the foam cells in atherosclerotic lesions are derived from phagocytic cells which are transported in the circulating blood, some rabbits with marked hypercholesteremia and early atherosclerotic lesions were subjected to intravenous injection of dilute india ink at two day intervals for three successive injections. Others were given intravenous injections of a neutral suspension of lithium carmine. In preparations of the tissues of the animals which had been given injections of india ink the phagocytic cells were laden with carbon granules but no carbon granules could be observed in the foam cells of the atherosclerotic lesions. In preparations of the tissues of the animals which had been given injections of lithium carmine abundant carmine granules were present in the cytoplasm of the phagocytic cells but none were observed in the foam cells of the atherosclerotic lesions.

In sections of the viscera of the animals given injections of india ink or of carmine, particularly in those of the spleen and the liver, carbon granules and carmine granules, respectively, were observed in many foam cells. These cells obviously represent phagocytic cells which have taken up lipid material and have become foam cells. Foam cells containing carbon or carmine granules have not been observed in the arterial lumens. Smears of the blood of rabbits which had been given injections of india ink or of lithium carmine, likewise, exhibited no cells which had taken up the injected substances. Free foam cells were observed in the lumen of an artery—the aorta—in only one of our animals. This animal had received injections of lithium carmine, but the cells in question had taken up none of the material. They probably represented foam cells which had become detached from an intimal plaque of the aorta. Our observations lend no support to the theory that phagocytic cells transported in the circulating blood constitute a source of the foam cells observed in atherosclerotic lesions.

*Genesis of Collagenous Tissue in Atherosclerotic Lesions*—Initial atherosclerotic lesions include no fibrous elements. Lesions of large arteries include some large collagenous fibers and some cells of connective tissue origin relatively early. The source of the cells in question undoubtedly is the meager connective tissue component of the intima. With development of the lesion the connective tissue elements increase in numbers. In rapidly growing intimal plaques nuclei of fibroblasts have been observed in amitotic division. Mitotic division of the nuclei of fibroblasts has not been observed. The collagenous nature of the fibrous elements has been determined by differential staining.

In instances in which the inner elastic membrane had broken down and the atherosclerotic lesion had extended into the media, collagenous fibers could be traced from the media into the intimal plaque. In sections through such lesions an increase in the number of fibroblasts in the media also was apparent.

### XANTHOMATOSIS

All the rabbits which had been fed a high cholesterol diet long enough for extensive atherosclerosis to have developed had xanthomatosis also. The tumors were associated mainly with synovial membranes and tendon sheaths. In all the tumors associated with the larger joints which have been carefully examined a definite relationship to the joint cavity could be demonstrated. In some of the animals xanthomatosis was widespread. Demonstrable tumors were present in relation to nearly all the joints of the extremities, the intervertebral articulations and the temporomandibular joints. It was not uncommon for tumors to extend along tendon sheaths. The tumors varied in size within a wide range. Many of the smaller ones were hardly large enough for gross demonstration. Some of the larger ones showed maximum dimensions of more than 5 cm. In general they were firm and tough, but when cut through they were found to include soft areas, from which a whitish, creamy substance could be expressed with slight pressure. Some were soft and contained small amounts of whitish fluid.

The histologic structure of these tumors resembled that of the intimal plaques of the atherosclerotic arteries (fig 5). They were composed mainly of masses of cells which were identical in appearance with the foam cells of the atherosclerotic lesions. A large lipid content of the tumors was demonstrated also by the use of fat stains. Some fibrous elements were constantly present between the masses of foam cells. In the more advanced tumors the fibrous tissue was more prominent than in the earlier ones. This fibrous tissue, like that in the arterial lesions, comprised mainly collagenous elements, as was demonstrated by differential staining. With the appearance of collagenous fibers, fibroblasts also became apparent in the tumor. As the collagenous tissue increased in volume, the groups of foam cells became more widely separated. Many of the foam cells also underwent reduction in size.

Our observations relative to the genesis of the foam cells in xanthoma are less complete than those relative to the genesis of the foam cells in the atherosclerotic lesion. Since the xanthoma cells appear to be identical in histologic structure with the foam cells of the atherosclerotic lesion, they probably have a similar origin. The earliest foam cells of xanthoma are located just beneath the mesenchymal epithelium of the synovial membrane. The foam cells of the early stage of xanthoma undoubtedly arise mainly from mesenchymal epithelial cells and probably histiocytes located in the synovial membrane. As the tumor becomes vascular, additional foam cells may be derived from the vascular endothelium. Amitotic division of young xanthoma cells also has been observed.

### ASSOCIATED VISCERAL LESIONS

In all the rabbits which showed advanced atherosclerosis, lesions comprising foam cells were present in the viscera, particularly in the liver, the adrenal glands, the spleen and the kidneys.

In the liver Kupffer cells and other endothelial cells became transformed into foam cells. The hepatic cells also became laden with lipid material to the extent that in ordinary preparations many of them presented the vacuolated appearance



characteristic of foam cells. Lipid material has been demonstrated in these cells also by the use of fat stains. Sections of the livers of some of the animals exhibited areas in which the hepatic cells had undergone necrosis.

The adrenal glands of cholesterol-fed rabbits became markedly enlarged. In some of the animals these glands were more than twice their normal size. The enlargement was due in part to distention of the lymphatic vessels, particularly of those of the medulla, with masses of foam cells and in part to hypertrophy of cortical cells caused by cytoplasmic storage of excessive amounts of lipid material. The loading of the cortical cells with lipid material appears earliest in the fasciculate zone. Later it becomes apparent also in the reticular and glomerular zones. In animals fed cholesterol 200 days or longer the lymphatic vessels of the adrenal medulla were widely distended with foam cells. These cells formed compact masses, some of which gave evidence of necrosis in their central areas. Since the endothelium of the lymphatic channels of the adrenal glands represents a site of active proliferation of reticuloendothelial cells, it may be assumed that the foam cells in these channels were derived from the endothelium.

As the lymphatic vessels of the adrenal medulla became packed with foam cells, many of the chromaffin cells disappeared. In the glands in which the greatest distention of the lymphatic vessels was observed, few chromaffin cells could be detected. Those which remained reacted only lightly to the basic stains. The reduction of the volume of chromaffin tissue undoubtedly results in a decrease of the output of adrenin.

In sections of the spleens of rabbits with atherosclerotic lesions, foam cells were present particularly in the venous sinuses of the red pulp. In most of the cholesterol-fed animals the red pulp was congested and much increased in volume. The white pulp occupied a relatively small percentage of the area of the section. Its appearance suggested an actual reduction of its volume, although the spleen was appreciably enlarged. Many of the cells which had become differentiated into foam cells in the red pulp had ingested fragments of red blood cells and other particulate matter, indicating their capacity for phagocytosis. Foam cell differentiation of large lymphocytes also could be observed. The foam cells of the spleen exhibited wider variations in size than those of the liver or those of the adrenal glands. They were not closely packed in the venous sinuses, although they were present in large numbers.

Sections of the kidneys of all the rabbits with atherosclerotic lesions induced by cholesterol feeding presented involvement of the renal tubules. In the early lesions some of the epithelial cells, particularly those of the limbs of Henle's loops, appeared finely vacuolated, owing to lipid material in the cytoplasm. These cells reacted only lightly to the ordinary stains. Foam cells, either single or in small groups, could be observed between renal tubules. In later phases of cholesterol feeding the renal lesions were larger. Increasingly larger numbers of epithelial cells gave evidence of lipid material in the cytoplasm. In some areas, particularly in the outer zone of the medulla, the epithelium of several adjacent tubules was necrotic, and crystals were apparent in the necrotic mass. Foam cells were present between the renal tubules at many points. In some instances the masses of foam cells were sufficiently large and compact to press adjacent tubules apart. Clumps of cells laden with lipid material appeared also in the lumens of some renal tubules. These cells appeared to be detached epithelial cells. In oblique sections of tubules columns of cells could be observed, in certain instances, extending from the intact epithelium into the lumen. The epithelial cells of some of the proximal convoluted tubules exhibited vacuolation of the cytoplasm. In these cells the accumulation of lipid material was less impressive than in those of the limbs of Henle's loops. No other definite pathologic changes have been observed in the renal cortex.

In the kidneys the foam cells probably represent mainly cells which have been derived locally from the vascular endothelium. The lesions observed in the kidneys were sufficiently extensive to impair renal function to a high degree.

#### AUTONOMIC GANGLIONS AND NERVES

In all the cholesterol-fed rabbits in which atherosclerosis was well advanced the sympathetic ganglions and nerves exhibited recognizable alterations. Both the ganglion cells and the neuroglial tissue of the ganglions reacted positively to fat

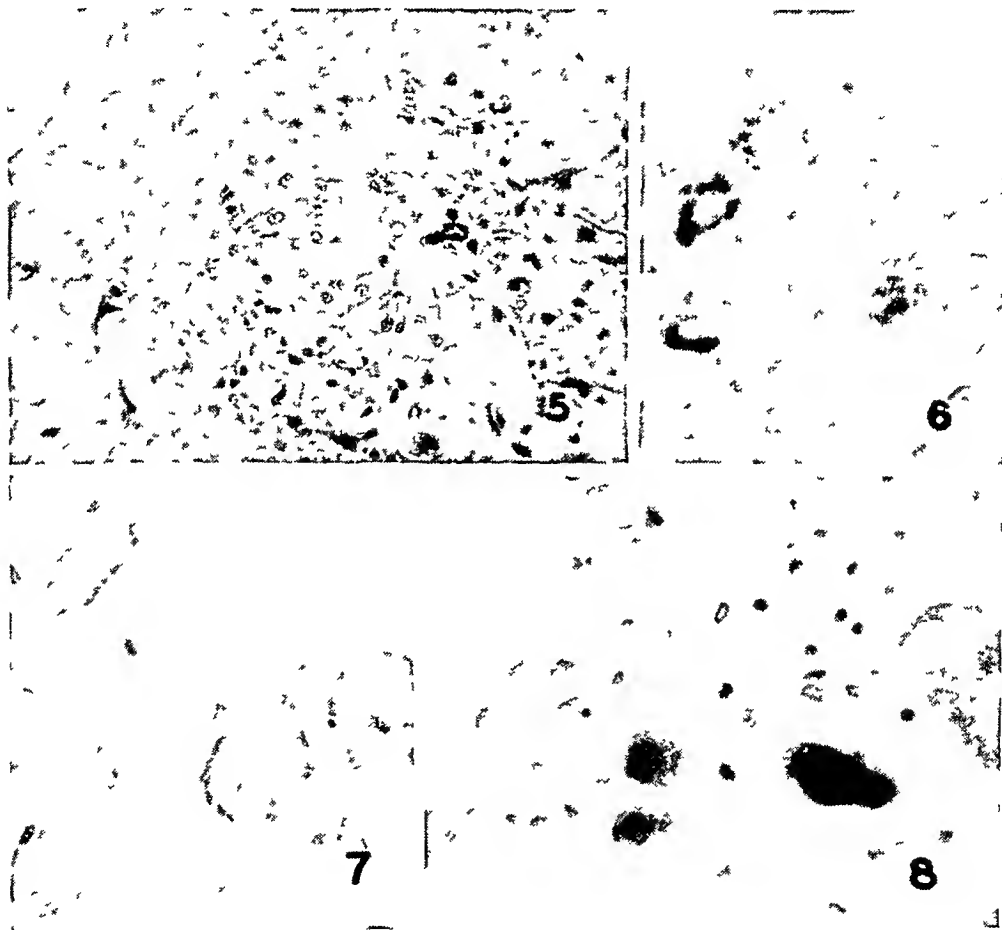


Fig 5—Section of a xanthoma

Fig 6—Section of an autonomic ganglion of a control rabbit

Fig 7—Section of an autonomic ganglion of a rabbit which had been fed cholesterol for 80 days

Fig 8—Section of an autonomic ganglion of a rabbit which had been fed cholesterol for 280 days

stains. Traces of lipid material, exclusive of myelin, were present also in the nerves. In sections which had been treated with fat solvents the cytoplasm of the ganglion cells was finely vacuolated, owing to extraction of its lipid content. Glycogen could not be demonstrated in the ganglion cells by the Bauer technic. The ganglion cells of control animals reacted positively to this technic. The chromidial content of the ganglion cells was diminished. The chromidial sub-

stance left in the cytoplasm was present mainly in the state of chromidial dust or in solution (figs 6, 7 and 8) The ascorbic acid content of the ganglion cells was undoubtedly diminished Many of the cells appeared to be entirely devoid of it In the ganglions of rabbits fed cholesterol 300 days or longer, most of the ganglion cells were shrunk to some degree and retained but little chromidial substance They were also devoid of glycogen and almost devoid of ascorbic acid The succession of alterations observed in the ganglion cells and their appearance in the later phases of cholesterol feeding suggest, not hyperactivity, but functional depression

#### COMMENT

The data obtained in this investigation indicate that rabbits fed a high cholesterol diet exhibit profound metabolic disturbances relatively early and that a condition of heterostasis persists throughout the period of cholesterol feeding Our data relative to the increased amount of lipid material in the blood and the elevation of blood pressure in cholesterol-fed rabbits agree in general with the data reported by other investigators who have studied these aspects of the problem Our data relative to the initiation of atherosclerotic lesions and their distribution in rabbits agree in general with those of Leary<sup>3</sup> and Wilens<sup>9</sup> Those relative to the origin of the foam cells support the theory that these cells arise locally mainly from the endothelial lining of the vessels This point of view has been advanced by Altschul<sup>10</sup> on the basis of studies of arteriosclerotic lesions in man It is supported also by data advanced by Moreton,<sup>7</sup> which indicate that lipid particles become lodged in the intima where they are taken up by cells, which become transformed into foam cells The assumption that phagocytic cells of reticuloendothelial origin which are transported in the circulating blood become differentiated into foam cells and invade the intima, advanced by Leary,<sup>3</sup> is incompatible with our findings

The similarity in cytologic structure of atherosclerotic plaques in man and in cholesterol-fed rabbits, particularly in the early phases of their development, has been emphasized by Anitschkow<sup>1</sup> Because of the high lipid content of the blood of the cholesterol-fed rabbits, the intimal plaques develop much more rapidly in the vessels of rabbits than in those of man Late lesions of man, consequently, cannot be compared directly with late lesions of experimental rabbits The former usually consist almost exclusively of fibrous tissue, owing to the extensive growth of collagenous fibers and the disappearance of the foam cells as the lipid material is resorbed In some instances, however, the differentiation of foam cells continues in atherosclerotic lesions in man even during the later stages of these lesions

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9 Wilens, S L. *Am J Path* 18 63, 1942

10 Altschul, R. *Arch Path* 38 305, 1944

A reduction of the number of foam cells, as well as of the sizes of many of those which remain, concurrent with an increase of the connective tissue components of the intimal plaque, is a prominent feature of atherosclerotic lesions in their later phases in cholesterol-fed rabbits. This is in full accord with the observation of Anitschkow<sup>1</sup> that almost complete fibrous transformation of atherosclerotic lesions may take place in experimental rabbits if the animals are kept alive two to three years after discontinuance of the cholesterol feeding. From the structural point of view, therefore, the atherosclerotic lesions of cholesterol-fed rabbits are essentially similar to the atherosclerotic lesions of man.

In the rabbit xanthomatosis appears to be due to the same causes as atherosclerosis. Our data relative to the initiation and the evolution of xanthoma in cholesterol-fed rabbits agree in general with those of Rusch, Bauman and Kline.<sup>11</sup> This tumor conforms closely in its cytologic structure to xanthoma of the tendon sheaths and the synovial membranes of man as described by Galloway and co-workers.<sup>5</sup> As far as our data have a bearing on the origin of the xanthoma cells, which appear to be identical with the foam cells of the atherosclerotic lesion, they support the assumption that these cells arise locally from the mesenchymal epithelium of the synovial membranes and probably from undifferentiated cells of the adjacent connective tissue.

The visceral tumors including foam cells which developed in cholesterol-fed rabbits also appeared to be due to the same causes as the atherosclerotic lesions. They did not play a primary role in the development of the atherosclerotic lesions. In view of the extent to which they developed in all the animals which had been fed cholesterol 90 days or longer, particularly in the liver, the spleen, the adrenal glands and the kidneys, they undoubtedly resulted in functional impairment of these organs in some degree. The paucity of the chromaffin tissue remaining in the adrenal medulla suggests material reduction of the output of adrenin. The extensive damage of the renal tubules also suggests extensive impairment of renal function. Impairment of renal function undoubtedly is a factor in the development of the hypertension and, consequently, in the atherosclerosis.

Our data relative to the vasomotor nerves do not indicate exaggerated vasomotor activity but indicate rather functional depletion of the vasomotor mechanisms due to faulty metabolism, including that of the sympathetic ganglion cells. If the output of adrenin is materially decreased, as is suggested by the reduction in the amount of chromaffin tissue in the adrenal medulla, this would tend further to reduce sympha-

11 Rusch, H. P., Bauman, C. A., and Kline, B. E. *Arch. Path.* **28** 163, 1939.

thetic tonus. Vasomotor nerve activity, consequently, cannot be regarded as a significant factor in the production and the maintenance of the elevated blood pressure of these animals.

#### SUMMARY

In rabbits fed cholesterol daily in addition to a rabbit chow hypercholesteremia and hypertension developed. In those fed 1 Gm of cholesterol daily atherosclerotic lesions appeared in the aorta and other large arteries within 30 days. As the process advanced lesions appeared also in small arteries. The initial atheroma consisted mainly of a few foam cells in the intima. As the number of foam cells increased, a thick intimal plaque was formed. Collagenous fibers soon appeared in the plaque. Associated with these fibers were some fibroblasts. The late lesion included a large percentage of fibrous tissue, including numerous fibroblasts. In sections the late intimal plaque exhibited stratification due to alternate arrangement of the fibrous tissue and the foam cells. Early atherosclerotic lesions were limited to the intima, many of the older lesions extended into the media.

The data support the assumption that the foam cells observed in atherosclerotic lesions are derived locally from the endothelium. The fibrous tissue is derived from connective tissue elements of the intima and the media.

Xanthomatosis occurred in all rabbits with advanced atherosclerosis. The data relative to the origin of the xanthoma cells support the assumption that these also arise locally from the mesenchymal epithelium and from undifferentiated cells of the synovial membranes. In rabbits with advanced atherosclerosis, lesions characterized by foam cells developed also in viscera, particularly in the liver, the spleen, the adrenal glands and the kidneys. There also the foam cells appeared to arise locally. The autonomic ganglions and nerves exhibited a succession of alterations which in the later phases of cholesterol feeding suggested functional depression of the cells.

# THE PHENOMENON OF LEUKERGY

LUDWIK FLECK  
AND  
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**L**EUKERGY is a phenomenon found in citrated blood (Fleck, 1942) which manifests itself as an agglomeration or clumping of leukocytes. The clumps contain up to 20 or more cells with marked tendency to cellular homogeneity. It appears in infectious diseases in man and animals, and it can be experimentally elicited by an intravenous injection of live or killed gram-negative bacteria (e g, *Bacterium coli*, *Salmonella typhi*, *Bacterium proteus* X) or by an intrapleural injection of turpentine. A report of our earlier studies on leukergy has been published.<sup>1</sup> The term "leukergy" is derived from three words of Greek origin *λευκός* *λύτος* (white cell) and *ἐργεῖν* to act.)

An improved technic was used, as follows

*Tube Test*—The sample of blood is drawn either from a vein or from the heart of the animal. It is then mixed with a 3.8 per cent sodium citrate solution in the proportion of 4:1 and incubated. After one, two, or three hours of incubation a drop of the well mixed blood is taken with a large platinum loop and placed on a slide as a large drop. The slide is rocked gently, dried in the incubator and either stained with Wright stain or fixed by heat and then stained with an aqueous solution of methylene blue. The smears may be hemolyzed. This is best done by placing the slides with the dried drops in the ice box for several minutes and subsequently exhaling several times on the cooled slides following their removal from the refrigerator. The condensing steam lyses the blood without washing off the drop. This technic differs from that previously described in that we use whole blood without attempting to concentrate leukocytes by either sedimentation or centrifugation.

*Drop Test*—A drop of a 2 per cent sodium citrate solution is thoroughly mixed with an equal drop of blood on a microscopic slide previously coated with a 1 per cent alcoholic solution of brilliant cresyl blue. The slide is then incubated for 15 to 20 minutes in a moist chamber and can be examined microscopically either with a dry lens or with oil immersion after drying of the drop followed by gentle rocking. Hanging drops of citrated blood with or even without brilliant cresyl blue observed on a heated microscopic table give also clearcut pictures of leukergy.

In figure 1, *A* and *B* show a positive and a negative drop test, and *C* a positive tube test, with remarkable homogeneity of the groups.

The results of the tube test are in general more reliable. The drop test is quick and simple.

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1. Fleck, L., and Murczynska, Z. *Texas Rep Biol & Med* 5:156, 1947.

## CYTOLOGIC SELECTIVITY OF LEUKERGY

The phenomenon shows two kinds of cytologic selectivity

The degree of agglomeration of various kinds of white blood cells is different i.e., the percentage of clumped leukocytes, lymphocytes or monocytes varies in different cases. In most instances the clumping involves mainly polymorphonuclear leukocytes

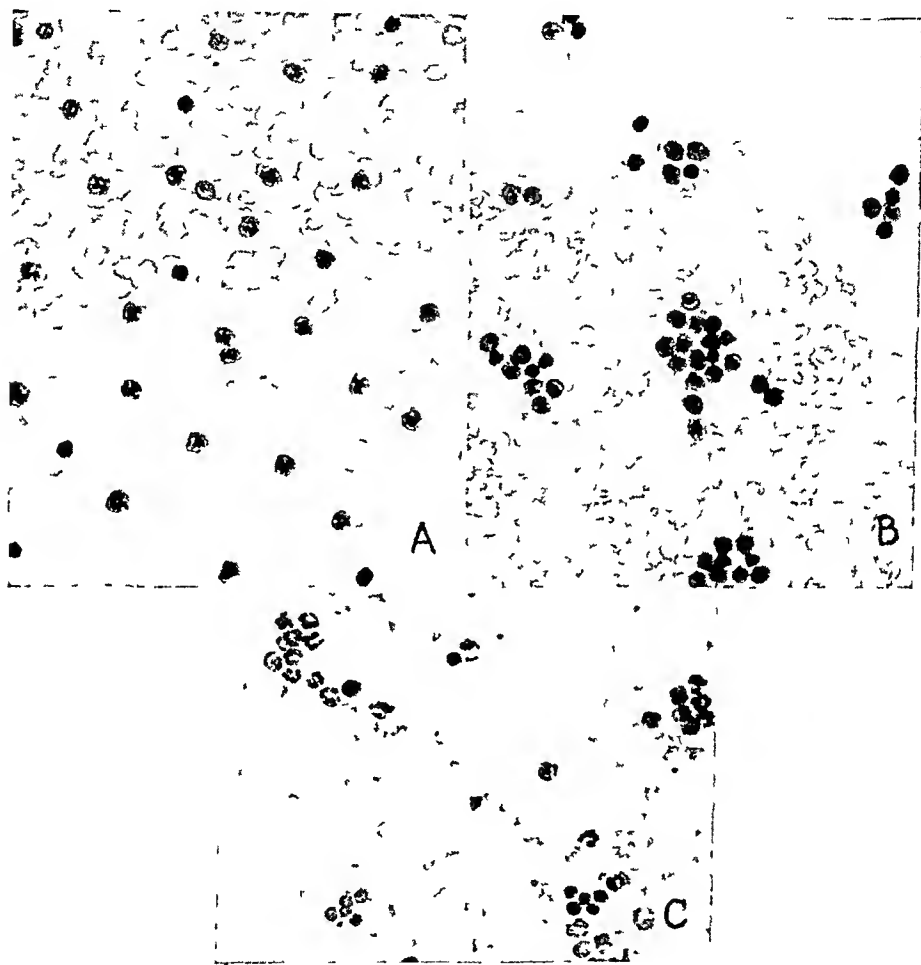


Fig 1—*A*, normal blood in drop test, *B*, leukergy in drop test, *C*, tube test of rabbit after intravenous injection of killed colon bacilli

There is a marked tendency to form cytologically homogeneous groups, i.e., groups containing either neutrophils, or lymphocytes, or monocytes

Both features of selectivity are well demonstrated in table 1, which gives the counts in a sample from a tube test stained with Wright stain in a case of toxic dermatitis, and in following tables. The inequality of the process, which involves 81 per cent of eosinophils and only 14 per cent of lymphocytes, is obvious. Larger groups (from 5 cells up)

contained 3 per cent lymphocytes and 39 per cent neutrophils, although the respective total numbers in the counted area of the smear were approximately equal. The nine larger groups consisted of 81 cells. They are shown in table 2.

TABLE 1—*Leukergy in Toxic Dermatitis, Case 1, Sample 1*

|   | Neutro<br>phils | Eosino<br>phils | Lympho<br>cytes | Mono<br>cytes | Total     |
|---|-----------------|-----------------|-----------------|---------------|-----------|
| Counted number of scattered and<br>agglomerated cells | 63 (29%)        | 90 (39%)        | 65 (28%)        | 9 (4%)        | 232       |
| In groups from 3 cells up                             | 49 (72%)        | 73 (81%)        | 9 (14%)         | 2 (22%)       | 133 (57%) |
| In groups from 5 cells up                             | 27 (39%)        | 50 (55%)        | 2 (3%)          | 2 (22%)       | 81 (34%)  |
| Scattered cells                                       | 19 (28%)        | 17 (19%)        | 56 (86%)        | 7 (78%)       | 99 (43%)  |

TABLE 2—*Composition of the Nine Larger Groups in Case 1, Sample 1*

|                    | Eosino<br>phils<br>Only | Neutro<br>phils<br>Only | Mixed<br>Groups | Total  |       |
|--------------------|-------------------------|-------------------------|-----------------|--------|-------|
|                    |                         |                         |                 | Groups | Cells |
| Groups of 5 cells  | 1                       | 1                       |                 | 2      | 10    |
| Groups of 6 cells  |                         |                         | 1               | 1      | 6     |
| Groups of 7 cells  | 1                       |                         | 1               | 2      | 14    |
| Groups of 10 cells |                         |                         | 1               | 1      | 10    |
| Groups of 11 cells |                         |                         | 1               | 1      | 11    |
| Groups of 13 cells |                         |                         | 1               | 1      | 13    |
| Groups of 17 cells |                         |                         | 1               | 1      | 17    |
| Total              |                         |                         |                 | 9      | 81    |

TABLE 3—*Leukergy in Toxic Dermatitis, Case 1, Sample 2*

|   | Neutro<br>phils | Eosino<br>phils | Lympho<br>cytes | Mono<br>cytes | Total     |
|---|-----------------|-----------------|-----------------|---------------|-----------|
| Counted number of scattered and<br>agglomerated cells | 72 (35%)        | 84 (41%)        | 39 (19%)        | 11 (5%)       | 206       |
| In groups from 3 cells up                             | 45 (62%)        | 73 (87%)        | 6 (15%)         | 8 (27%)       | 127 (62%) |
| In groups from 5 cells up                             | 29 (40%)        | 48 (57%)        | 5 (13%)         |               | 82 (40%)  |
| Scattered cells                                       | 27 (38%)        | 11 (13%)        | 33 (85%)        | 8 (73%)       | 79 (38%)  |

TABLE 4—*Composition of the Ten Larger Groups in Case 1, Sample 2*

|                    | Eosino<br>phils<br>Only | Neutro<br>phils<br>Only | Mixed<br>Groups | Total  |       |
|--------------------|-------------------------|-------------------------|-----------------|--------|-------|
|                    |                         |                         |                 | Groups | Cells |
| Groups of 5 cells  | 1                       | 1                       |                 | 2      | 10    |
| Groups of 6 cells  |                         |                         | 2               | 2      | 12    |
| Groups of 7 cells  | 1                       |                         | 2               | 3      | 21    |
| Groups of 11 cells |                         |                         | 1               | 1      | 11    |
| Groups of 13 cells |                         |                         | 1               | 1      | 13    |
| Groups of 15 cells |                         |                         | 1               | 1      | 15    |
| Total              |                         |                         |                 | 10     | 82    |

In a second drop of blood taken simultaneously in the same case we found the numbers given in table 3.

The conformity of both results is fairly good, at least in respect to neutrophils and eosinophils. The larger groups (from 5 cells up) in this preparation contained 82 cells. They are shown in table 4.



The total of nineteen groups (9 + 10) from 5 cells upward contained 163 cells

|             |                 |       |      |
|-------------|-----------------|-------|------|
| Eosinophils | 50 + 48 cells = | 98 =  | 60%  |
| Neutrophils | 27 + 29 cells = | 56 =  | 34%  |
| Others      | 4 + 5 cells =   | 9 =   | 6%   |
|             |                 | 163 = | 100% |

All four groups of 5 cells were homogeneous. If the number of cytologically homogeneous groups depended only on the percentage of this special kind of cells available for agglomeration (i.e., if there were no tendency to form homogeneous groups), then the expected frequency of homogeneous 5 cell groups in this case would be

For eosinophils  $\left[\frac{60}{100}\right]^5 = 0.08 = 8\% \pm 13$  and it is 50 per cent

For neutrophils  $\left[\frac{34}{100}\right]^5 = 0.0045 = 0.45\% \pm 3.1$  and it is 50 per cent<sup>2</sup>

Of five 7 cell groups, 2 were pure eosinophil groups, which makes 40 per cent. The expected number (if one assumes that there is no tendency to form homogeneous groups) would be

$\left[\frac{60}{100}\right]^7 = 0.028 = 2.8\% \pm 7.3$  and it is 40 per cent

The frequency of homogeneous groups surpasses the expected frequency by more than three times the cited standard deviation. Almost pure homogeneous groups of 11, 15 or 17 cells accentuate still further the tendency to cytologic homogeneity.

TABLE 5—*Leukergy in Typhus Fever, Case 2, Eighth Day*

| Counted number of scattered and agglomerated cells | Neutrophils | Lymphocytes | Monocytes | Total     |
|--|-------------|-------------|-----------|-----------|
| In groups from 3 cells up                          | 154 (72%)   | 46 (22%)    | 12 (6%)   | 212       |
| In groups from 5 cells up                          | 145 (94%)   | 20 (43%)    | 10 (83%)  | 175 (83%) |
| Scattered cells *                                  | 145 (94%)   | 20 (43%)    | 7 (58%)   | 172 (81%) |
|  | 9 (6%)      | 26 (57%)    | 2 (17%)   | 37 (17%)  |

In table 5, which gives the counts in a case of typhus fever, one finds leukergy higher than in case 1 (83 against 57 per cent), with marked agglomeration of lymphocytes and monocytes (e.g., 43 against 14 per cent). Large groups from 5 cells up in the counted area are shown in table 6.

The lymphocytes formed homogeneous groups only one of 7, and another of 13 cells, although only 11 per cent of the total number of cells clumped in groups of 5 or more were lymphocytes. This coincidence cannot be explained as purely fortuitous.

2 Dr Steinhaus, professor of mathematics, University of Wroclaw, and Dr Biernacki, professor of mathematics, University of Lubin, helped us in the statistical evaluation of our results.

In case 5 (table 9) a separate count of thirty major groups (from 5 cells upward) with 214 cells gave the picture shown in table 10

TABLE 6—Composition of the Thirteen Larger Groups in Case 2

|                    | Neutrophils<br>Only | Lymphocytes<br>Only | Monocytes<br>Only | Mixed<br>Groups | Total  |       |
|--------------------|---------------------|---------------------|-------------------|-----------------|--------|-------|
|                    |                     |                     |                   |                 | Groups | Cells |
| Groups of 5 cells  | 2                   |                     | 1                 |                 | 3      | 15    |
| Groups of 6 cells  |                     |                     |                   | 1               | 1      | 6     |
| Groups of 7 cells  | 2                   | 1                   |                   |                 | 3      | 21    |
| Groups of 9 cells  |                     |                     |                   | 1               | 1      | 9     |
| Groups of 13 cells |                     | 1                   |                   |                 | 1      | 13    |
| Groups of 14 cells | 1                   |                     |                   |                 | 1      | 14    |
| Groups of 19 cells | 1                   |                     |                   |                 | 1      | 19    |
| Groups of 33 cells | 1                   |                     |                   |                 | 1      | 33    |
| Groups of 42 cells | 1                   |                     |                   |                 | 1      | 42    |
| Total              |                     |                     |                   |                 | 13     | 172   |

TABLE 7—Leukergy in a Rabbit After Injection of Killed Colon Bacilli, Case 3

|  | Neutrophils | Lymphocytes | Monocytes | Total     |
|--|-------------|-------------|-----------|-----------|
| Counted number of scattered and agglomerated cells | 36 (19%)    | 141 (73%)   | 16 (8%)   | 193       |
| In groups from 3 cells up                          | 10 (28%)    | 41 (29%)    | 14 (87%)  | 65 (34%)  |
| In groups from 5 cells up                          | 6 (17%)     | 25 (17%)    | 12 (75%)  | 93 (22%)  |
| Scattered cells                                    | 26 (72%)    | 100 (71%)   | 2 (13%)   | 128 (66%) |

TABLE 8—Leukergy in Typhoid Fever, Third Week, Case 4

|  | Neutrophils | Lymphocytes | Monocytes | Total     |
|--|-------------|-------------|-----------|-----------|
| Counted number of scattered and agglomerated cells | 114 (65%)   | 47 (27%)    | 14 (8%)   | 175       |
| In groups from 3 cells up                          | 103 (90%)   | 18 (38%)    | 10 (71%)  | 129 (74%) |
| In groups from 5 cells up                          | 76 (66%)    | 13 (27%)    | 7 (50%)   | 94 (53%)  |
| Scattered cells                                    | 11 (10%)    | 29 (62%)    | 4 (29%)   | 46 (26%)  |

TABLE 9—Leukergy in Fibrocaceous Pulmonary Tuberculosis, Case 5  
(Temperature, 37.8 C [100 F], Erythrocyte Sedimentation Rate,  
96 mm in First Hour and 120 mm in Second Hour)

|  | Neutrophils | Lymphocytes | Monocytes | Eosinophils | Total     |
|--|-------------|-------------|-----------|-------------|-----------|
| Counted number of scattered and agglomerated cells | 168 (71%)   | 58 (25%)    | 5 (2%)    | 4 (2%)      | 235       |
| In groups from 3 cells up                          | 109 (65%)   | 21 (36%)    | 2 (40%)   | 3 (75%)     | 135 (57%) |
| Scattered cells                                    | 59 (35%)    | 37 (64%)    | 3 (60%)   | 1 (25%)     | 100 (43%) |

TABLE 10—Composition of Thirty Major Groups in Case 5

|                    | Neutrophils<br>Only | Lymphocytes<br>Only | Monocytes<br>Only | Mixed<br>Groups | Total  |       |
|--------------------|---------------------|---------------------|-------------------|-----------------|--------|-------|
|                    |                     |                     |                   |                 | Groups | Cells |
| Groups of 5 cells  | 5                   | 2                   |                   | 1               | 8      | 40    |
| Groups of 6 cells  | 2                   |                     |                   | 5               | 7      | 42    |
| Groups of 7 cells  | 1                   | 2                   | 2                 | 3               | 8      | 56    |
| Groups of 8 cells  |                     |                     |                   | 4               | 4      | 32    |
| Groups of 10 cells |                     |                     |                   | 1               | 1      | 10    |
| Groups of 12 cells | 1                   |                     |                   |                 | 1      | 12    |
| Groups of 22 cells |                     |                     |                   | 1               | 1      | 22    |
| Total              |                     |                     |                   |                 | 30     | 214   |

Those groups contain 117 (54 per cent) neutrophils, 64 (29 per cent) lymphocytes, 30 (14 per cent) monocytes and 4 eosinophils. Nine groups are pure neutrophil groups of 5 or more cells, which makes 30 per cent, and the expected number would be (according to Dr Biernacki)

$$f(p) = \frac{1}{30} (8p^6 + 7p^6 + 8p^7 + 4p^8 + p^{10} + p^{12} + p^{22})$$

where

$$p = \frac{54}{100}, \text{ which is } 54 \text{ per cent}$$

The standard deviation is determined as follows

$$\text{Standard deviation} = f^1(p) \sqrt{\frac{pq}{n}} < 1 \text{ per cent}$$

where

$$f^1(p) \text{ is the derivative of } f(p), p = \frac{54}{100}, q = \frac{46}{100} \text{ and } n = 214$$

The result is that the theoretic frequency of pure neutrophil groups is 23 per cent  $\pm 1$ , whereas the observed frequency is 30 per cent. A similar count shows that the theoretic frequency of pure lymphocytic groups is 0.09 per cent  $\pm 0.07$ , and the observed frequency is 13 per cent. The tendency toward homogeneity of the groups is clear.

The clinical value of leukergy counts (percentage of agglomerated cells of each kind) has to be proved. Observations by clinicians interested in testing this new method of blood examination might be of importance.

The results of our preliminary examinations<sup>3</sup> may be summarized as follows:

Blood of normal persons and animals tested by the described method shows no agglomeration of leukocytes.

Different inflammatory stimuli and febrile diseases incite leukergy. It also was found in infants several weeks old.

Leukergy is not always accompanied by leukocytosis, it may be seen also in leukopenia.

It is not necessarily accompanied by fever, we see it in convalescents with normal temperature.

In some diseases with high erythrocyte sedimentation rates leukergy was only slight (chronic nephritis, neoplastic cachexia, panmyelophthisis). In others with marked leukergy the erythrocyte sedimentation rates were normal (convalescence after infectious diseases, febrile allergic states).

3 Fleck, L., and Borecka, D. *Ann Univ M Curie Skłodowska* 1:335, 1946

- Irradiation with high doses of roentgen rays failed to incite leukergy in rabbits. The same observation was made after ultraviolet irradiation.

## LEUKERGY AND PHAGOCYTOSIS

We assumed that leukergy may perhaps have an influence on phagocytosis. Experiments in rabbits failed to substantiate this view.

Immediately after the injection of bacteria which induce leukergy, the phagocytosis of injected bacteria diminishes. This negative phase is followed by a rise after a few days and the phagocytic index reaches a level higher than before the injection. The course of leukergy is

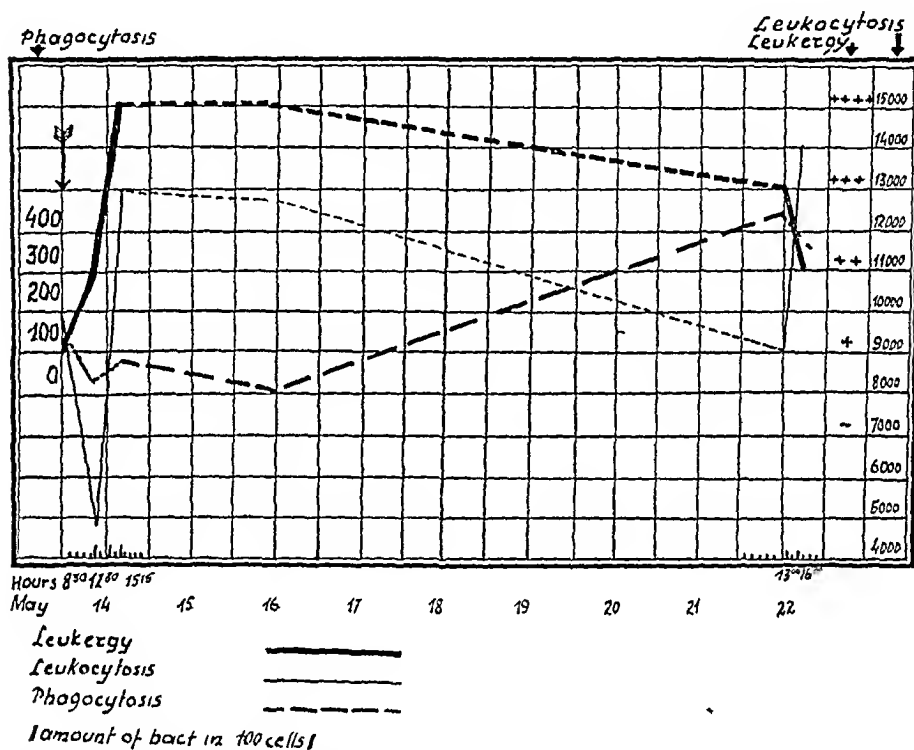


Fig. 2—Leukocytosis, leukergy and phagocytosis (represented respectively by a thin black line, a heavy black line and a broken line) in a rabbit after intravenous injection of killed colon bacilli.

different. It rises several hours after the injection of bacteria and drops in a few days. Figure 2 illustrates one such experiment.

The negative phase of phagocytosis is probably due to the binding of opsonins by the injected antigen, according to the recent results of Boivin, Delaunay and Pages<sup>4</sup>. This follows from the observation that leukocytes studied during the negative phase when mixed with serum taken before the injection give a phagocytic index as high as that before

<sup>4</sup> Boivin, A., Delaunay, A., and Pages, J. Bull. Acad. de med., Paris **128**: 305, 1944.

the injection. The rise in phagocytic activity several days after injection is caused by the increase in the titer of opsonins. The following experiments may serve to illustrate these relations.

#### EXPERIMENT 1

Rabbit 35, seven days after intravenous injection of killed colon bacilli  
leukergy ++++

Rabbit 37, normal leukergy ±

Leukocytes of 35 + serum of 35 314 bacteria in 100 cells

Leukocytes of 35 + serum of 37 178 bacteria in 100 cells

Leukocytes of 37 + serum of 37 310 bacteria in 100 cells

Leukocytes of 37 + serum of 35 840 bacteria in 100 cells

#### EXPERIMENT 2

Rabbit 42, seven hours after intravenous injection of killed colon bacilli  
leukergy ++++

Leukocytes of 42 + serum of 42 (both taken seven hours after injection)  
74 bacteria in 100 cells

Leukocytes 42 (seven hours after injection) + serum of 42 (before injection)  
163 bacteria in 100 cells

In any case the agglomerated leukocytes in leukergic blood do not show any more phagocytosed bacteria than the scattered leukocytes occurring in the same smear. There seems to be, however, another possibility of relating leukergy and phagocytosis. A great number of bacteria adhere to clumps of blood platelets, for in inflammation almost all the bacteria stick to these elements. The increased agglutination of platelets in inflammation might be compared to the action of anti-bacterial agglutinins which appear only later, bacteria are fixed and thus localized. As leukergic leukocytes adhere to a high degree to agglutinated platelets, an indirect influence of leukergy on phagocytosis might be assumed, i.e., if agglutinated platelets acted in the inflamed area as a factor facilitating the contact between leukocytes and bacteria. Under conditions of the experiment, where the number of bacteria is sufficiently high to provide easy contact, this effect cannot be seen.

It must be stressed, however, that the process of leukergy is independent of the presence of platelets. The following experiment may prove it.

A rabbit was given an injection of an antiplatelet serum obtained by immunizing a guinea pig with rabbit blood platelets. Ten minutes after the intravenous injection of 2 cc of the antiplatelet serum smears of the rabbit's blood showed practically no platelets. The number before the injection was 30 per thousand red blood cells. Simultaneously the rabbit received intravenously a killed suspension of *B. proteus* X<sub>10</sub> and the next day showed a high degree of leukergy. Microscopic examination revealed large groups of leukocytes free of platelets. At the time the number of platelets (these appeared gigantic in size in most cases) was 8 per thousand red blood cells. This slight rise was probably due to the inflammatory stimulus given by the injection of bacteria.

## SOME FACTORS PROVOKING EXPERIMENTAL LEUKERGY

Leukergy may be easily induced in rabbits by injection (particularly intravenous injections) of gram-negative bacteria (*B. coli*, *S. typhi*, *B. proteus* X<sup>10</sup>). Approximately 150 millions of killed bacteria are sufficient. Gram-positive cocci (a strain of *Staphylococcus albus*, a strain of *Staphylococcus aureus* from a human abscess and a strain of *Streptococcus hemolyticus*) have been much less efficient. Attempts to produce leukergy by injections of killed *Staph. aureus* (up to 2 billion micro-organisms) failed. A suspension of live *Staph. albus*, however, injected into the knee joint was followed by purulent arthritis with leukergy. Injections of killed streptococci up to 500 millions had no effect on the leukergy. Not less than 2 billions of killed streptococci were needed to provoke leukergy. Killed diphtheria bacilli in amounts up to 500 millions were injected without effect. Two billions gave only a moderate and a short lasting leukergic effect.

Injections of the endotoxin of *B. coli* (antigen glycolipoidal) prepared in accordance with the technic of Boivin with trichloroacetic acid gave high leukergy. The hapten obtained by splitting the endotoxin<sup>5</sup> after boiling with acetic acid gave either no leukergy or at most a weak reaction, even though the material was injected in amounts twice or three times the original amount of endotoxin employed<sup>6</sup>.

Horse serum (1 to 2 cc) gave in rabbits either no leukergy or only slight degrees, though in previously sensitized animals the reaction was sometimes strong, simultaneously with the appearance of the Arthus phenomenon.

Continued experiments can be summarized as follows:

Following perenteral administration of milk to men or animals, a transient leukergy of several hours' duration has been observed.

Heparin causes clumping of white blood cells *in vivo* and *in vitro*. The clumps show a tendency to form cytologically homogeneous groups, their homogeneity being much like that observed in leukergic blood. The phenomenon is now under investigation.

Furthermore, leukergy has been observed in the last five months of uncomplicated human pregnancy, persisting for some time after normal

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5 The endotoxin and the hapten were controlled by precipitation with a specific serum. Rabbits given injections of the endotoxin show a rise in their respective agglutinating titers, rabbits given injections of the hapten failed to show a rise.

6 In comparison with the *B. coli* endotoxin the substance obtained from a strain of *Staph. aureus* by the method of Boivin with trichloroacetic acid was wholly ineffective.

delivery Observations have been made on human pregnancy by Dr Kwiatkowski and confirmed by us on pregnant rabbits

Other kinds of bacteria or bacterial products will be investigated

#### COMMENT

The most striking feature of leukergy is the cytologic selectivity of the process of cell clumping The agglomeration itself can be seen at first sight in microscopic preparations, but the selectivity must be statistically confirmed to avoid any misleading formations of accidental groups Statistical counts quoted show that the frequency of homogeneous groups surpasses by far the frequency of random agglomerations This holds true as well for the agglomeration of neutrophil leukocytes, which is most common, as for eosinophils, lymphocytes or monocytes It leads to the assumption that there are special mechanisms aggregating similar cells

The study of different leukergy counts gives the impression that white blood cells of the kind which is just increasing in number are most susceptible to agglomeration, i e , neutrophil leukocytes in increasing neutrophil leukocytosis, and lymphocytes in increasing lymphocytosis Leukergy lasts approximately four days after a single stimulus (e g , injection of killed bacteria)

The forces causing the selective agglomeration may be physicochemical in nature, or they may represent the special kind of physicochemical factors which are termed serologic forces In the latter case leukergy would be a case of autoagglutination The assumption of a serologic mechanism in leukergy is based on the fact that the homogeneous clumps consist of cells possessing the same specific, serologically distinguishable antigens, as stressed in a previous report At present we may add the observation of a case of acute myeloid leukemia, in which myeloblasts formed separate groups, whereas more mature granulocytes, beginning with myelocytes, gave mixed clumps A specific antigen of myeloblasts was found by Fleck and Lille<sup>7</sup> in 1940 and was confirmed in later studies by Steinberg and Martin<sup>8</sup>

It must be emphasized, however, that other, nonserologic mechanisms cannot be excluded, since the formation of cytologically homogeneous agglomerations of white blood cells may be compared to the grouping of other blood cells—e g , rouleaux formation of erythrocytes or clumping of thrombocytes, probably phenomena of a nonserologic type

Our attempts to find differentiating features between leukergy and isoagglutination or heteroagglutination of red blood cells by means of different salt concentrations failed a 2 per cent sodium chloride solution represses a high degree of leukergy and checks completely a slight one

7 Fleck, L, and Lille, F Am Rev Soviet Med 3 174, 1945

8 Steinberg B, and Martin, R A J Immunol 52 71, 1946

A 4 per cent solution checks even a high leukergic effect. In general, the same holds true for isoagglutination of human red blood cells, and for the agglutination of rabbit white and red blood cells by either normal or immune antirabbit serum of rats and dogs.

Not all pyrogenic procedures have the same effect in producing leukergy, i.e., the injection of milk or foreign serum has either no effect or only a weak one. Foreign serum seems to act only on sensitized animals and even then not regularly. Injection of killed streptococci, staphylococci or diphtheria bacilli gives only slight leukergic reactions, in contrast to gram-negative bacteria, such as *B. coli*, *S. typhi* or *B. proteus*, which produce as a rule high degrees of leukergy. The antigen, glycolipoidal in nature, of colon bacilli prepared after Boivin's method gives the same positive result, but the hapten obtained by boiling this antigen with acetic acid does not produce marked leukergy. At present the question cannot be answered whether leukergy appearing after intrapleural injection of turpentine is due to the direct action of turpentine or to bacterial contamination of inflammatory foci in the lungs, which always have been found at autopsy.

The high occurrence of leukergy in infectious diseases and the regular appearance of leukergy in the experiment seems to allow us to look on it as on a phenomenon with a distinct rôle in pathogenesis. It has no direct relation to phagocytosis, the possibility of an indirect influence on phagocytosis through contact with thrombocytes was mentioned in an earlier paragraph. The influence on the migration of leukocytes, a problem of actual importance in the light of recent investigations of Boivin and Delaunay, will be studied.

#### SUMMARY

Leukergy, the phenomenon consisting in clumping of leukocytes in cytologically homogeneous groups, shows two kinds of cytologic selectivity.

- 1 The degree of agglomeration of various kinds of white blood cells is different, i.e., the percentage of clumped leukocytes, lymphocytes or monocytes varies in different cases. The clumping of neutrophils is most frequent. A differential method of evaluating the degree of leukergy is described.
- 2 The groups of cells show a remarkable cytologic homogeneity. This can be statistically proved.

Leukergy has no direct influence on phagocytosis. An indirect influence through the close contact of leukergic leukocytes with clumps of platelets, including bacteria, is discussed.



Leukergy can be positively induced by injection of gram-negative bacteria or their endotoxins. The effect of gram-positive bacteria is much weaker and less definite.

Increased salt concentration depresses the agglomeration of leukocytes, this action being similar to that on isoagglutination and heteroagglutination of blood cells.

## CHANGES IN THE CAPSULE OF THE LYMPH NODE IN EXPERIMENTAL HYPERPLASIA

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THE QUESTION as to how the capsule of the lymph node accommodates the enlargement of the organ during hyperplasia has not been answered. The solution may lie in one or more of the following possibilities: that the capsule, by means of proliferative changes, keeps pace with internal enlargement, that it is stretched passively as though it were an elastic sac, or that it disintegrates partially or totally and is later restored with new tissue which delimits the periphery of the enlarged organ.

The numerous publications concerning experimental and clinical observations of lymph node hyperplasia are concerned with the structural changes of the lymphatic tissues exclusive of the capsule. In morphogenetic studies the capsular changes have been mentioned by a few investigators, but as incidental findings. Gulland,<sup>1</sup> after making comparative studies of mammalian nodes, stated that the unequal peripheral growth of the cortical lymphatic tissue formed nodules protruding into the capsule, so that the segment of the latter between two points of evagination became a trabecula. Similar observations were reported by Kling<sup>2</sup> with regard to the developing nodes of human lungs. Sabin,<sup>3</sup> in studying the development of lymph nodes in pig fetuses, concluded that the capsule impeded peripheral enlargement of the organ. She observed growth only at the margins where the capsule was still deficient. These points of view seem to indicate that the capsule, once formed, does not undergo any significant reconstruction throughout the growth of the organ.

The capsule, according to current views, is a fibrous structure which completely invests the node and through which the afferent lymphatic vessels enter the subcapsular sinus. It is said to be thickest at the hilus, where it ensheathes nodal blood vessels, nerves and efferent lymphatic vessels. From the inner surface of the capsule, including that of the hilus, strands of connective tissue pass into the organ as the trabecular framework. Although the thickness of the capsule varies with age and species, its structural components consist predominantly

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1 Gulland, G. L. *J. Path. & Bact.* **2**: 447, 1893.

2 Kling, C. A. *Arch. f. mikr. Anat.* **63**: 575, 1904.

3 Sabin, F. *Am. J. Anat.* **4**: 355, 1905.

of collagenous connective tissue fibers, with small amounts of reticular and elastic fibers. In certain animals (e.g., the cow), the capsules of the lymph nodes are especially thick and contain, in addition to the connective tissue component, an abundance of smooth muscle fibers.

In this paper, attention is focused entirely on the changes in the capsule of hyperplastic lymph nodes. Among the several laboratory animals convenient for experimentation the hamster was chosen first because its small nodes could be sectioned serially with economy of time and material. Later the rat was used because its nodes, although slightly larger, possess more definite and thicker capsules. Finally, normal and hyperplastic lymph nodes of calves were included in the study because the capsules of the nodes of these animals are the thickest and the most complex.

#### MATERIALS AND METHODS

Fifteen adult hamsters were given injections of Eberthella typhosus vaccine (0.1 cc injected subcutaneously into each of the hindpaws). Four animals were killed after twenty-four hours, 4 were killed after seven days, in 7 animals the same dosage was repeated every three days, and the animals were killed ten days after the third injection. Four normal adult hamsters served as controls. The right and left popliteal and inguinal lymph nodes of both the vaccine-treated and the control animals were excised immediately after the killing.

Seventeen adult rats received 0.2 cc of E. typhosus vaccine into each of their paws. Three were killed after nine hours, 4 after twenty-four hours, 2 after forty-eight hours and 8 after seventy-two hours. Seven normal adult rats served as controls. The right and left popliteal, inguinal and axillary lymph nodes of vaccine-treated and control animals were removed immediately after the killing.

Mediastinal and bronchial lymph nodes of 6 calves with acute pulmonary infections (bronchopneumonia) were obtained from the slaughterhouses through the aid of Dr. L. J. Cook, Meat Inspection Division, United States Department of Agriculture, Chicago. Mediastinal, bronchial and mesenteric nodes from healthy calves served as controls.

The specimens from hamsters and rats were fixed in 4 per cent formaldehyde solution, embedded in paraffin, sectioned serially at approximately 10 microns and stained with either hematoxylin-eosin or hematoxylin-picric-fuchsin. The calf nodes, already fixed in formaldehyde solution when received, were prepared for histologic study in the same manner.

#### OBSERVATIONS

In general, the unfixed extirpated nodes of the vaccine-treated hamsters and rats appeared large and hyperemic. Since, however, nodes which appeared unusually large were occasionally found among the control specimens, a search for the possible significance of dimensional differences was not attempted. Furthermore, preliminary experience had revealed that microscopic differentiation was the most reliable, if not the only, means of determining whether or not hyperplasia was present.

*Hamsters*—In the popliteal and inguinal nodes of normal hamsters several layers of fibroblasts dispersed between collagenous fibers formed a thin but intact capsule over the entire periphery of the organ. At the hilus, however, this

capsule became areolar and merged imperceptibly with the loose perinodal tissue. As a result, the boundary of the parenchyma at the hilus was not sharp, for here the perinodal tissue contained free lymphocytes that had migrated from the medulla. Sections stained with hematoxylin-picrofuchsin failed to show any muscular tissue in the capsule and the trabeculae.

The lymph nodes of vaccine-treated hamsters all exhibited hyperplasia of varying intensities. Even twenty-four hours after injection of the vaccine, large numbers of lymphocytes filled the sinuses, and the contrast between the cortex and the medulla so characteristic of the normal organ was effaced. Secondary nodules were not as yet present, and under low magnification the entire section of the node appeared as a dense, homogeneous mass which was markedly basophilic. The striking feature which typified these specimens was that the capsule not only was thin, being only the thickness of a single cell in places, but showed areas of disintegration. Figure 1 portrays a section of an inguinal node, in which the capsule overlying the cortex is distinct up to point *r*, but between this point and the hilus (*h*), the parenchyma merged directly with the perinodal adipose tissue (*f*). Free lymphocytes migrating peripherally into the latter produced a ragged cortical margin. In the hilar region the medulla sent out numerous small tongues of lymphocytes and reticular cells into the areolar tissue.

The hyperplastic changes just described were more pronounced in the nodes of hamsters killed seven days after the injection of vaccine. The areas in which cortical lymphocytic tissue protruded through open gaps in the capsule were more extensive. Both the extracapsular protrusions and the cortex proper had numerous pale-staining secondary centers in the lymphatic nodules. More marked, too, were the massive amounts of the medulla that projected into the areolar tissue of the hilus. In the perinodal adipose tissue just peripheral to the intact portions of the capsule there were occasional microscopic islands of lymphocytes, a feature which was not noticed in animals killed twenty-four hours after vaccination.

The nodes of hamsters which were given triple injections and killed ten days later showed essentially the same hyperplastic changes that were found in animals killed one week after a single dose.

*Rats*—The normal somatic lymph nodes of the rat, except for their greater size, resembled those of the hamsters in their general histologic features. Although the capsule consisted of several tiers of fusiform fibroblasts as in the hamster, its thickness was about twice as great because of the increase of coarse collagenous fibers. Toward the hilus the compact capsule was again replaced by areolar tissue (fig 2). This area was infiltrated by the medullary lymphatic tissue to a more pronounced extent than in the hamster, so that the determination of the external contour of the organ in the hilar region was difficult.

The nodes from the rats killed nine hours after injection of the vaccine appeared normal. In a few specimens the sinuses were filled with acidophilic coagulum which contained lymphocytes. The capsule was thin but intact, and the areolar tissue of the hilus contained small nests of reticular cells among the infiltrating lymphocytes.

With longer postinjection periods, the histologic changes simulated those which characterized the hyperplastic nodes of the hamsters. The extending of a portion of the cortex through a deficiency in the capsule was most prevalent in rats killed twenty-four hours after vaccination. Figure 3 shows a

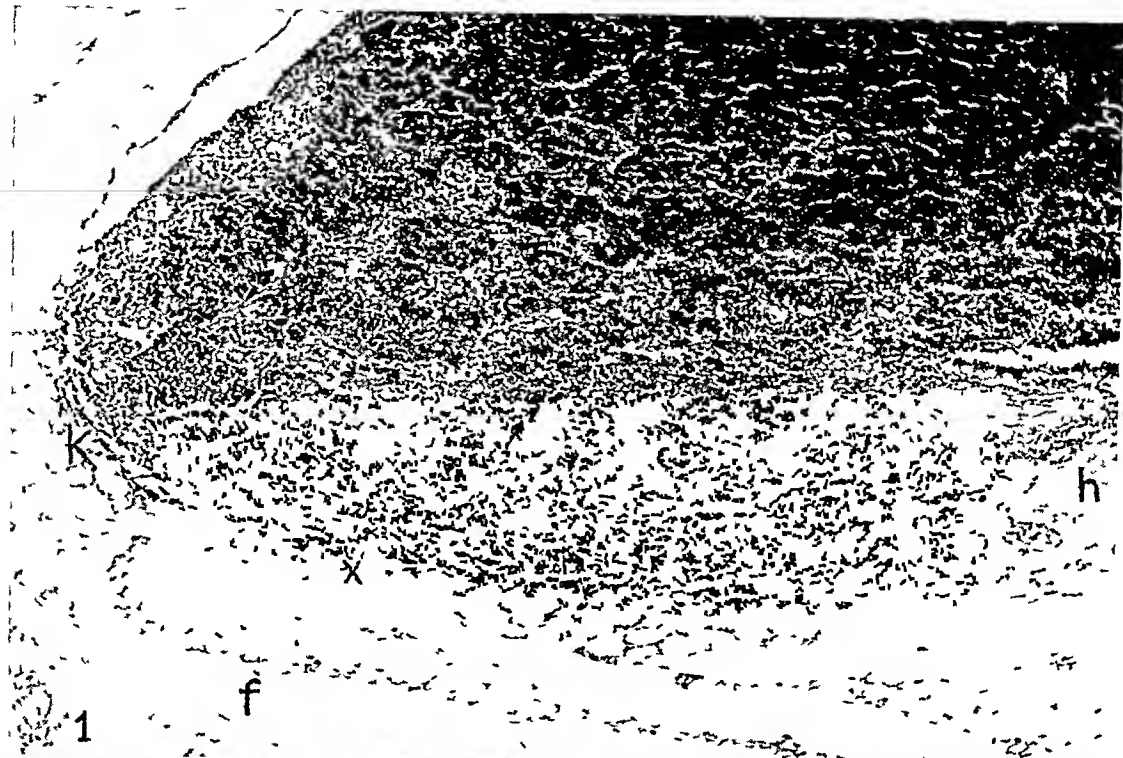


Fig 1—Section through a hyperplastic inguinal lymph node (adult hamster killed twenty-four hours after injection of typhoid vaccine),  $\times 97$ . The capsule, *k*, which covers the pole of the node at the left ends at *r*. Note that between *r* and the hilus, *h*, the capsule is absent and the periphery of the cortex is in direct contact with perimodal areolar tissue, *f*.

Fig 2—Section through the hilar region of a normal popliteal lymph node (adult rat, control),  $\times 95$ . *c*, indicates cortex, *h*, hilus, *el*, efferent lymphatic vessel, *m*, medulla, *lm*, lymphocytes infiltrating the hilus from the medulla. Note the contrast in sharpness between the peripheral margin of the cortex and that of the medulla.

Figures 1 to 6, inclusive, are unretouched photomicrographs

section of a relatively small popliteal node in which approximately one quarter of the surface was devoid of capsule. The most peripheral extensions of the cortex consisted of lymphocytes (*l*) scattered diffusely in the perinodal fat. Centrally, however, there was a dense zone of reticular cells with abundant cytoplasm which resembled the primitive mesenchymal cells of a developing lymph node. Few lymphocytes were dispersed among them. The contrast between the eosinophilic reticular layer (*r*) and the basophilic cortex proper (*c*) was sharp, with a distinct boundary between the two (broken line in figure). The massive peripheral protruding of the medulla into the areolar tissue of the hilus was a common occurrence in these specimens.

In the hyperplastic nodes of rats killed forty-eight to seventy-two hours after vaccination, the morphologic contrast between the original cortex and the newly proliferated, extracapsular lymphatic tissue (indicated by arrows in fig 4) was less, for the latter now contained an abundance of lymphocytes. The proliferated tongue possessed a sharp peripheral boundary, which was demarcated from the perinodal adipose tissue by a thin cap of fibroblasts lying parallel to the surface (*kl*). It is interesting to note that this newly formed covering was continuous above with the remnant of the original capsule (*k*) while below it joined the hilar areolar tissue. The hilus, however, still showed a diffuse scattering of medullary tissue.

*Calves*—The normal visceral lymph nodes of calves are large oblong or bean-shaped bodies, which on the average measured about 2.5 by 1 cm. In overall histologic appearance they resembled the nodes of the hamster and the rat. The capsule, however, was thick and consisted chiefly of compact alternating strata of collagenous connective tissue and smooth muscle fibers. The latter were abundant also in both the coarse and the fine trabeculae of the cortex and the medulla. At the hilus the capsule broke up into an areolar structure in which connective tissue elements, smooth muscle and adipose tissue lay without definite arrangement. The outer margin of the medulla was again indistinct because the lymphocytes diffusely infiltrated the hilus.

Most of the bronchial and mediastinal lymph nodes of animals condemned at the abattoir because of bronchopneumonia displayed hyperplastic changes, while a few, despite their large size, appeared normal when examined microscopically. In the hyperplastic ones the outstanding manifestation was the presence of small areas of partial destruction of the capsule overlying the cortex. Yet unlike the hyperplastic nodes of hamsters and rats, these did not have cortical tissue protruding through minute breaks in the capsule. The typical appearance of incomplete loss of the capsule is shown in figure 5. The inner capsular surface was rough and scalloped, owing to a row of discrete spaces (*s*) which were lined with a single layer of flattened cells. The spaces found at the right of the figure were empty except for small amounts of coagulum and cell debris, while those toward the left communicated with the underlying subcapsular sinus and were packed with cells. The latter were lymphocytes and free reticular cells which extended peripherally from the congested subcapsular sinus (*ss*). It should be emphasized that the spaces just described were totally independent of the intracapsular afferent lymphatic channels as proved when they were traced through serial sections. The capsule here, as a result of undermining by the bayou-like arrangement, was about one-half the average thickness and also showed structural differences. Lymphocytes and reticular cells infiltrated between the layers of fibroblasts and muscle fibers and formed small intracapsular islands (*i*). Although not shown in the figure, the perinodal adipose tissue at this site contained similar accumulations.

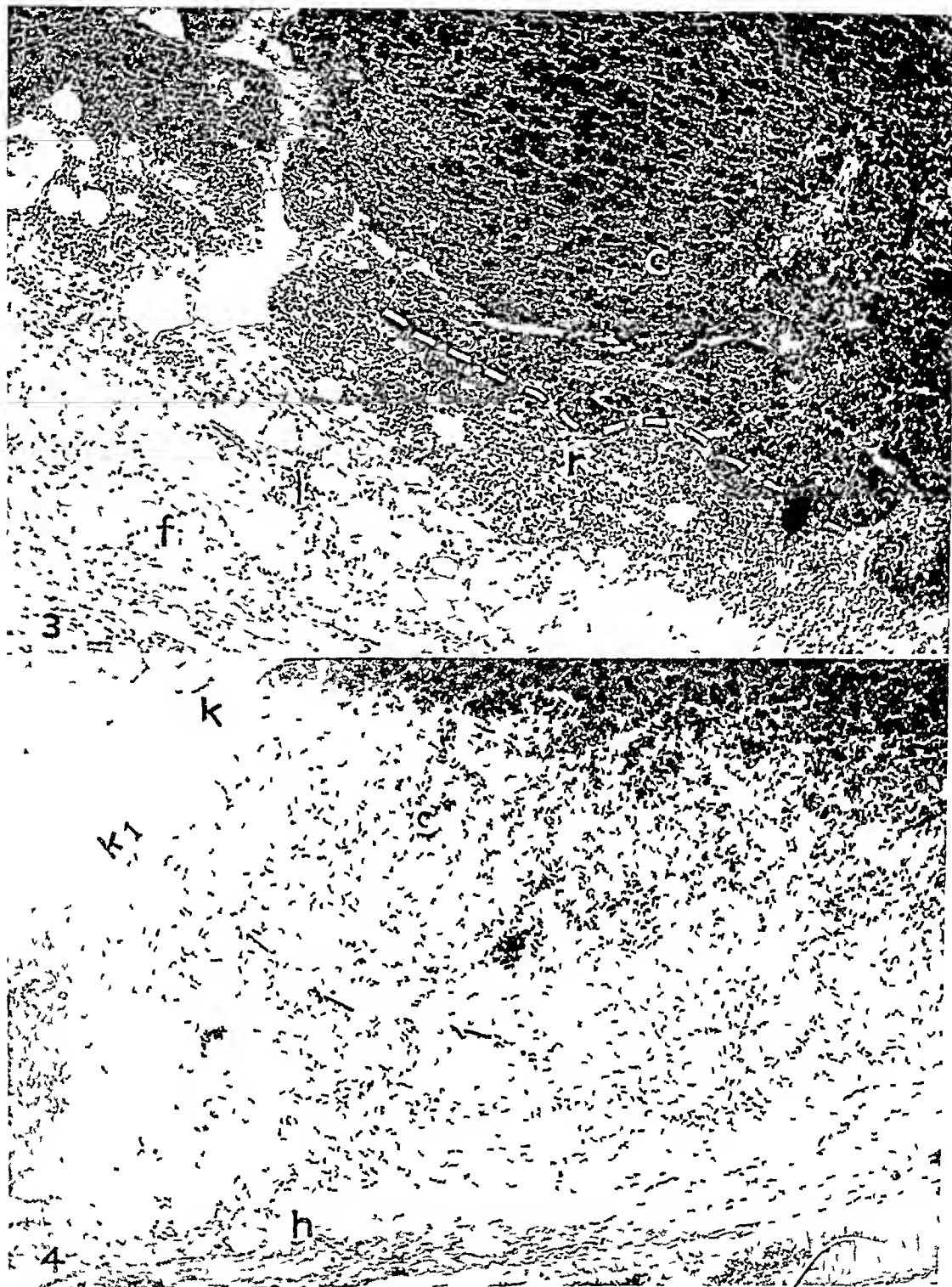


Fig 3—Section through an area of extracapsular protrusion of the cortex in a hyperplastic popliteal lymph node (adult rat killed twenty-four hours after injection of typhoid vaccine),  $\times 95$  *c*, indicates cortex, *f*, perinodal adipose tissue infiltrated by lymphocytes, *l, r*, solid mass of reticular cells and lymphocytes which is located just peripheral to a site of disintegration of the capsule. The broken line indicates the original boundary of the cortex.

Fig 4—Section through an area of extracapsular protrusion of the cortex in a hyperplastic axillary lymph node (adult rat killed seventy-two hours after injection of typhoid vaccine),  $\times 95$  *c*, indicates cortex, *k*, original capsule covering the surface of the cortex, *kl*, condensation of fibroblasts which forms a cap over the peripheral margin of newly proliferated lymphatic tissue, *h*, hilus of node diffusely infiltrated by lymphocytes. Arrows indicate the probable course of the extracapsular migration of the hyperplastic cortex.



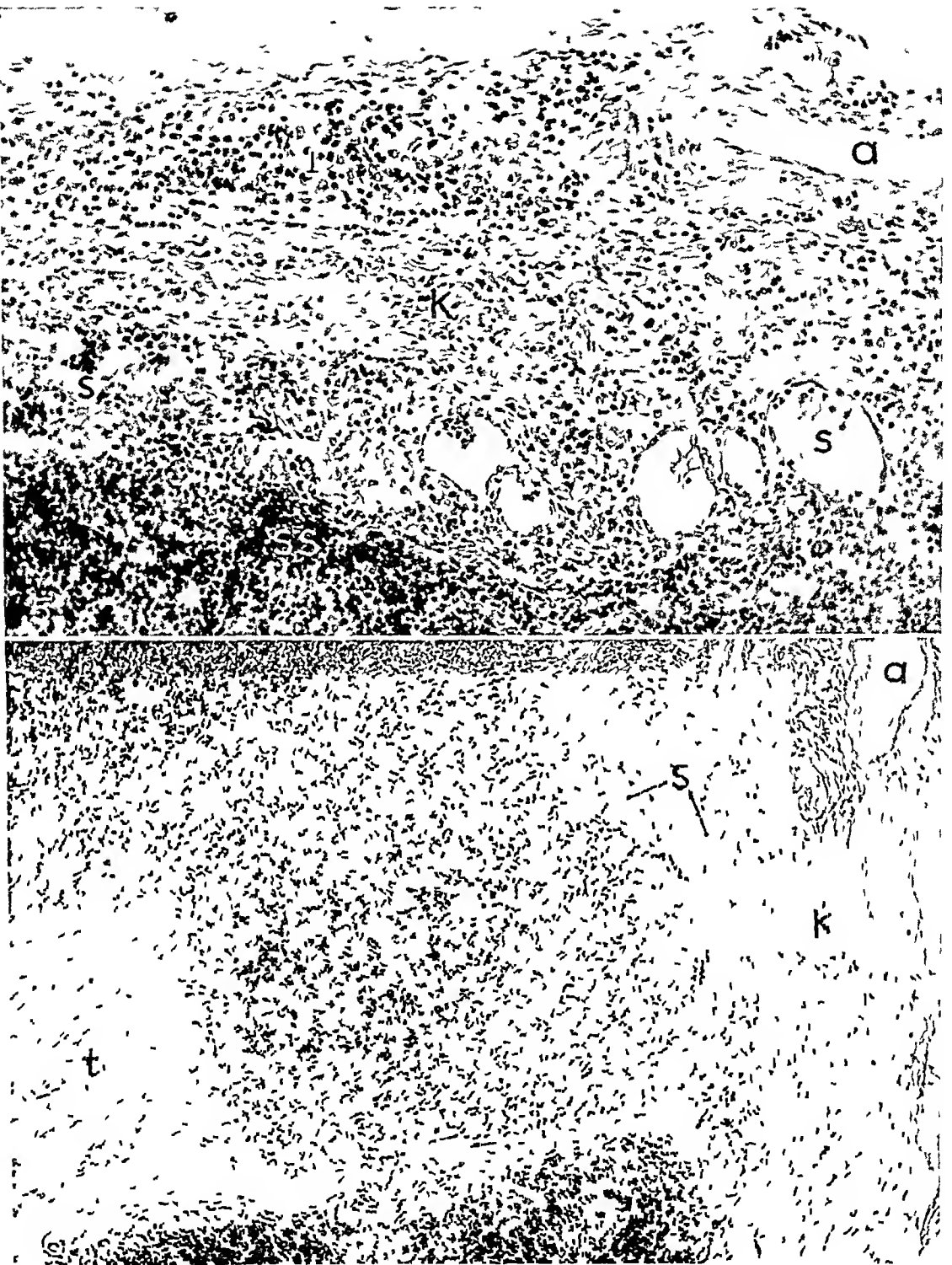


Fig 5—Section through an area of partial resorption of the capsule of a hyperplastic bronchial lymph node (calf condemned at abattoir for bronchopneumonia),  $\times 200$  *ss*, indicates a subcapsular sinus crowded with lymphocytes and free reticular cells, *s*, blind spaces lined with squamous epithelium, *k*, thinned capsule infiltrated by lymphocytes and reticular cells, *t*, intracapsular island of lymphocytes and reticular cells, *a*, afferent lymphatic channel

Fig 6—Section through an area of trabecular and capsular resorption in a hyperplastic bronchial lymph node (calf condemned at abattoir for bronchopneumonia),  $\times 90$  *c*, indicates cortex, *k*, thinned capsule showing blind spaces, *s*, along its inner surface, *a*, afferent lymphatic channel. Note the solitary lymphatic nodule which occupies the site at which the capsule connects with the trabecula, *t*



The surfaces of the trabeculae situated adjacent to sites where the capsule was being eroded were also affected in a like manner (fig 6) The section passes through the greatest diameter of a large solitary lymph nodule which had almost cut across the entire thickness of the trabecula The thinned capsule, as well as the remaining body of the trabecula, was heavily infiltrated with lymphocytes and reticular cells

Finally, the changes in the hilar region were almost identical with those observed in the hyperplastic nodes of hamsters and rats The medulla extended into the hilus as dense cords and islands Farther peripherally, the loose tissue filling the hilus contained diffusely scattered lymphocytes

#### COMMENT

The possibility that the node enlarges in hyperplasia by breaking out peripherally through the capsule seems to be supported by the present experiments

In the hyperplastic nodes of hamsters, the portions of the cortical lymphatic tissue protrude through the openings in the capsule and infiltrate the perinodal adipose tissue Despite the range of postinjection periods (twenty-four hours to ten days) the results are fairly consistent, the slight difference being a quantitative one in which the degree of extracapsular migration of the lymphatic tissue increases with longer experimentation There is no indication that a new capsule is formed around the protruded mass At the hilus of the normal node of the hamster the capsule becomes loose and merges with the areolar tissue Even in the normal node, lymphocytes of the medulla diffusely infiltrate the hilus to some degree It is here that the maximum peripheral expansion of the node is noticed in hyperplasia, for the areolar tissue offers probably less resistance than the capsule over the cortical surface

The findings are almost duplicated in the hyperplastic lymph nodes of rats, although the capsule of the normal organ of this animal is somewhat thicker than that in the hamster Again the greatest amount of peripheral enlargement occurs in the areolar tissue of the hilar region A feature of the specimens from the rats which was not encountered in the hamsters is the quality of the structural changes in the capsule over the cortex incident with the duration of the postinjection period In nodes of animals killed twenty-four hours after vaccination the protruded lymphatic tissue lies in direct contact with the perinodal fat, but in the animals killed after forty-eight to seventy-two hours such areas show the beginning of what appears to be a new capsule which delimits the extent of peripheral hyperplasia This probably signifies a cessation of further enlargement and a restoration or repair of the damaged part of the capsule The fibroblasts of the new segment are derived from the indifferent cells of either the perinodal adipose tissue or the reticular tissue of the cortex

Thus, in nodes of hamsters and rats, acute hyperplasia means that portions of the cortex have penetrated through the thin capsule and the medulla has spread peripherally into the hilus. Consequently, the enlarged node possesses a rough, "bumpy" external contour over the cortical surface, while the familiar indentation at the hilus is mostly obliterated.

In the calf's lymph node, the capsule of which is much thicker, capsular rupture apparently does not occur, at least in the hyperplastic material observed. Areas are found, however, in which the capsule is eroded along its inner surface with consequent thinning. Lymphocytes and free reticular cells emigrate from the subcapsular sinus beyond the area of erosion, as witnessed by numerous intracapsular and extracapsular islands of these cells. That the capsule and the trabeculae are labile and subject to modification under proper stimulus is shown by the severe trabecular resorptions. To explain the absence of extracapsular projections of the cortical lymphatic tissue of the hyperplastic nodes of calves tempts one to indulge in speculations. Has the node enlarged by commensurate interstitial proliferation of the capsule? Since the material obtained from the slaughterhouse gives no clue as to the time of initiation or the duration of the hyperplastic changes, the chance that these specimens exhibit terminal stages in which capsular damages have been repaired must be considered. Over the medulla at the hilus the enclosure is completed by a loose structure, and it is at this site that pronounced peripheral enlargement of lymphatic tissue takes place in hyperplasia.

That acute hyperplasia of the node involves passive stretching of its covering seems to be a minor consideration, especially when the structural features of the capsule are appreciated. It may be parenthetically stated that the general question as to how a saclike structure, whether it be a capsule of a viscus or the skin of the body surface, responds to fluctuations of the masses contained within has not received a satisfactory explanation.

#### SUMMARY

The normal somatic lymph nodes of hamsters and rats possess a capsule which is relatively thin, compact and devoid of smooth muscle. At the hilus the capsule is areolar and contains lymphocytes which have infiltrated it from the medulla of the node. In hyperplasia the capsule shows areas of disintegration through which portions of the cortex extend out into the perinodal adipose tissue. Simultaneously large amounts of the medulla migrate peripherally as cords and islands into the surrounding areolar tissue of the hilus.

The capsules of the normal visceral lymph nodes of calves are extremely thick and contain abundant smooth muscle. However, at the hilus the medulla is covered by a loose structure which resembles areolar tissue, except that it exhibits strands of scattered smooth muscle, and contains lymphocytes that have emigrated from the medulla. In the hyperplastic nodes the capsule of the cortex is resorbed in certain areas from its inner surface. The portion of the capsule just peripheral to the resorption is thin and contains small islands of lymphocytes and reticular cells. The destructive process also affects any trabecula located near the site of capsular erosion. Disintegration involving locally the entire thickness of the capsule has not been found. The hilar region shows a massive migration of medullary parenchyma which extends into the surrounding loose tissue.

# DISTRIBUTION OF THE LYMPHATICS OF THE HUMAN KIDNEY AS SHOWN IN A CASE OF CARCINOMATOUS PERMEATION

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ALTHOUGH the lymphatic channels of the kidney have been studied by a number of investigators, and the distribution of the main channels is fairly well known, there is still no general agreement concerning the distribution of the smaller channels. This lack of agreement may be attributed to technical difficulties. Hitherto, with one exception, the distribution of the renal lymphatic channels has been traced by means of injections of pigment. As pigment introduced into the hilar trunks by retrograde injections is usually unable to pass the valves, the most successful studies have been those employing subcapsular injections particularly in living animals. It is apparent, however, that any forceful injection of pigment will tend to produce artefacts, which may make morphologic interpretation difficult and uncertain. The one study which did not involve injection of pigment was that of Vogel,<sup>1</sup> who studied a kidney in which the lymphatic channels were distended with carcinoma cells. It cannot be denied, however, that artefacts may likewise occur in such preparations.

The principal points of disagreement are concerned with the relations existing between the lymphatic channels and the glomeruli, the medulla and the medullary rays. With regard to the glomeruli, some authors<sup>2</sup> have believed that lymphatic vessels accompany the afferent and efferent arterioles, while others<sup>3</sup> have denied this, some<sup>4</sup> have believed that lymphatic channels enter the glomerulus, but others<sup>5</sup> have

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From the Laboratory of Pathology, School of Medicine, University of Pennsylvania

1 Vogel, L. Virchows Arch f path Anat **125** 495, 1891

2 (a) Rindowski, T, cited by Pierce<sup>3a</sup> (b) Kumita Arch f Anat u Physiol, 1909, p 99

3 (a) Pierce, E C, II Anat Rec **90** 315, 1944 (b) Vogel<sup>1</sup>

4 (a) Maximow, A A, and Bloom, W A Textbook of Histology, ed 3, Philadelphia, W B Saunders Company, 1938 (b) Ssysganow, A N Ztschr f d ges Anat **91** 770, 1930 (c) Rindowski<sup>2a</sup> (d) Kumita<sup>2b</sup>

5 (a) Stahr, H Arch f Anat u Entwcklngsgesch, 1900, p 41 (b) Kutsuna, M, Kiyozumi, M, and Yamasita, S, cited by Pierce<sup>3a</sup> (c) Drinker, C K, and Yoffee, J M Lymphatics, Lymph, and Lymphoid Tissue Harvard University Monograph in Medicine and Public Health No 2, Cambridge, Mass, Harvard University Press, 1941 (d) Pierce<sup>3a</sup>

been in disagreement. Some<sup>6</sup> have demonstrated the presence of extensive lymphatic networks about Bowman's capsule, whereas others<sup>7</sup> have failed to find them. With regard to the medulla, some authors<sup>8</sup> have reported the presence of lymphatic networks, while others<sup>7</sup> have asserted that lymphatic channels do not occur in this location. The same question arises concerning the presence or the absence of lymphatic channels in the medullary rays. In view of this controversy it seems worth while to trace the distribution of lymphatic channels in a human kidney obtained at a postmortem examination in a case in which extensive carcinoma had prominently permeated the lymph channels.

#### METHODS

The kidney studied was from a patient at the Hospital of the University of Pennsylvania who was found to have a large ulcerated adenocarcinoma in the cardia of the stomach, on the lesser curvature just below the esophagogastric junction. There was extraordinarily widespread lymphatic permeation extending to the kidneys, lungs, pancreas, adrenal glands and retroperitoneal tissues, the liver was not involved. No tumor nodules had formed, at all sites the neoplastic cells were confined to the lymphatic vessels.

The kidneys were bisected in the usual manner. As there was no gross evidence of carcinomatous involvement, the capsules were stripped from them to allow examination of the surface, hence the capsular lymphatic channels could not be included in the present study. Microscopic examination of routine sections revealed that the lymphatic vessels were made clearly visible because they were distended by seemingly solid cords of tumor cells within their lumens. The walls of the lymphatic channels and their endothelial lining were clearly demonstrated.

It was recognized, of course, that channels might exist of such small caliber that carcinomatous permeation might not be permitted. This study, therefore, is of necessity limited to the tracing of those channels which were large enough to contain carcinoma cells. In order to trace the course of the lymphatic vessels in detail, two longitudinal and two sets of consecutive coronal wedge-shaped blocks were cut as shown in fig. 1A. Several sections were prepared from each of the coronal blocks, and serial sections 5 microns in thickness were made from the longitudinal blocks. Fifty consecutive sections were cut from each of the latter, each section being examined. All tissues were stained with hematoxylin and eosin, the neoplastic cells were conspicuous because of their large size and basophilic affinity.

#### RESULTS

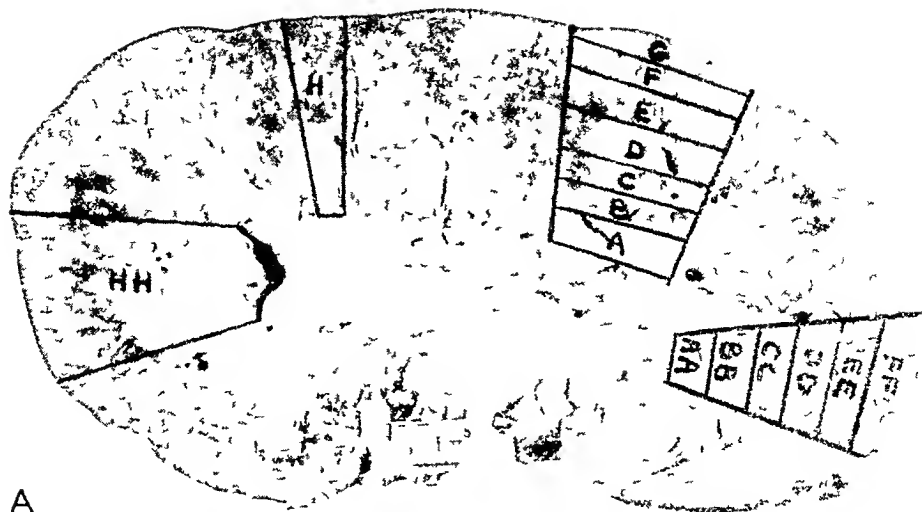
The distribution of the lymphatic channels of the human kidney examined is diagrammatically shown in figure 2. All the demonstrable lymphatic vessels are in close approximation to the arterial and venous channels, except the afferent and efferent arterioles and the glomeruli,

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6 Vogel<sup>1</sup> Rindowski<sup>2a</sup> Kumita<sup>2b</sup> Ssysganow<sup>4b</sup>

7 Stahr<sup>5a</sup> Kutsuna and others<sup>5b</sup> Pierce<sup>3a</sup>

8 Rindowski<sup>2a</sup> Kumita<sup>2b</sup> Ssysganow<sup>4b</sup>



A

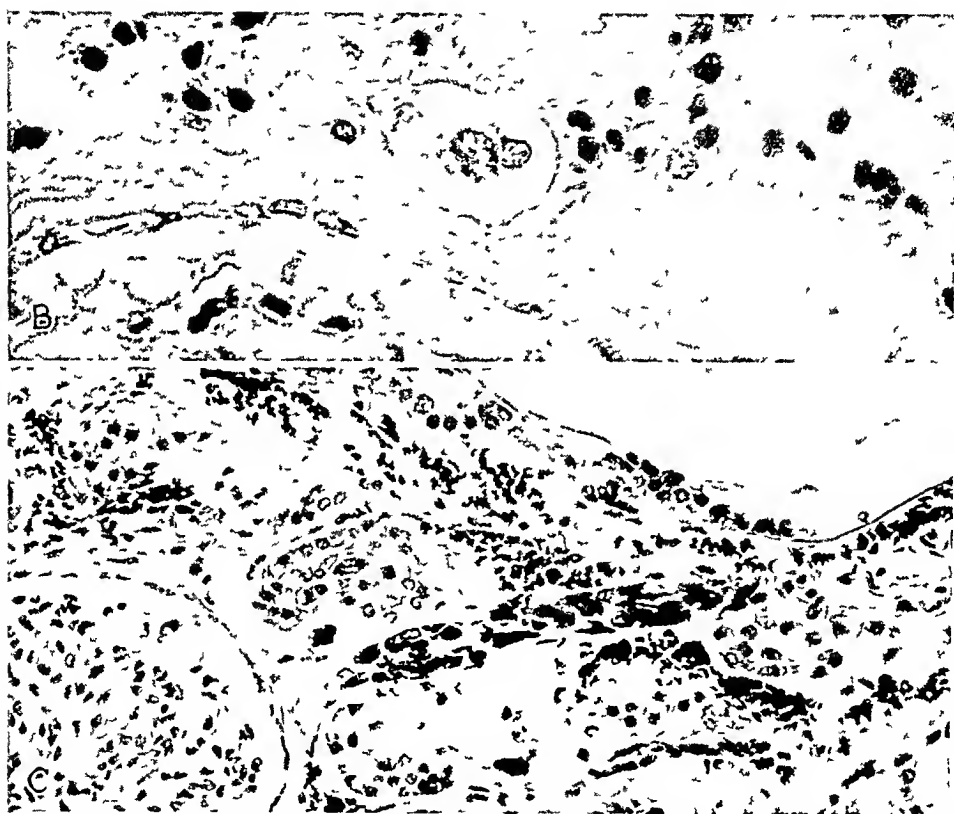


Fig 1—A, location of blocks of tissue studied. Sections A to G (inclusive) and AA to FF (inclusive) are coronal blocks. Sections H and HH are longitudinal blocks.

B, small lymphatic vessel lying just outside of Bowman's capsule. Serial sections show that the vessel begins here as a blind channel and continues as a short unbranched segment which communicates with the perivascular lymphatic networks (see fig 3 C). Note the close, but chance, relation of the lymphatic vessel to a convoluted tubule. Hematoxylin and eosin,  $\times 547$ .

C, lymphatic vessel appearing to enter the glomerulus at the lower left. Serial sections, however, show that it actually passes around a small arc of Bowman's capsule, and as part of an extensive network about the interlobular vein at the upper right. Hematoxylin and eosin,  $\times 265$ .

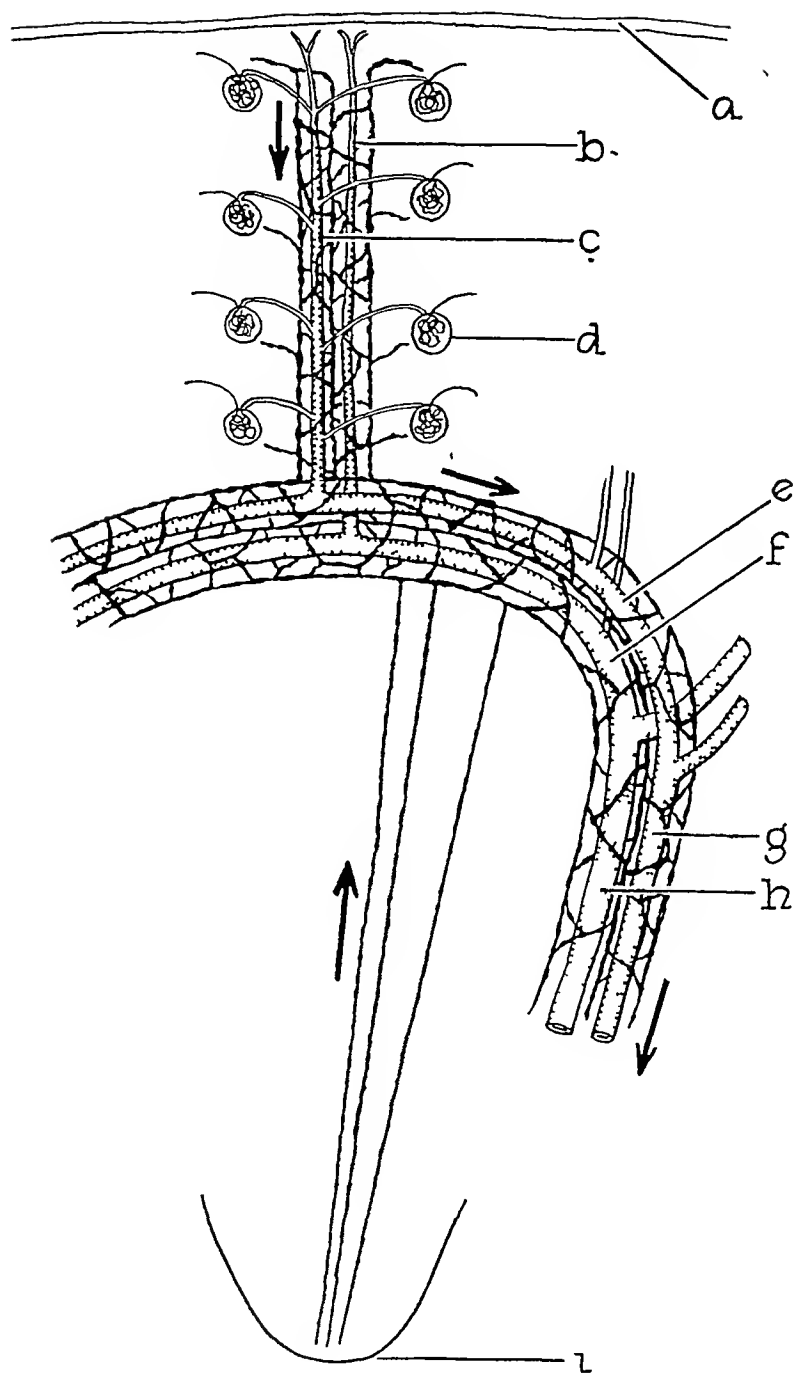


Fig 2—Lymphatic channels of the human kidney (diagrammatic) Two separate systems are demonstrable. One begins in the cortex and accompanies the interlobular vessels toward the corticomedullary junction, the other starts at the papilla and ascends to join the cortical system at the corticomedullary junction. From there large trunks follow the arcuate and interlobar vessels to leave the kidney at the hilum. Arrows show the probable direction of the lymph flow. The structures shown are (a) tunica fibrosa, (b) interlobular vein, (c) interlobular artery, (d) glomerulus, (e) arcuate artery, (f) arcuate vein, (g) interlobar artery, (h) interlobar vein and (i) papilla.



Fig 3—*A*, lymphatic vessel (cut longitudinally) in close contact with Bowman's capsule and forming a short arc about it. Serial sections show that this is actually a segment of a loose perivenous network accompanying an interlobular vein. Hematoxylin and eosin,  $\times 547$ .

*B*, two prominent lymphatic vessels lying on either side of an interlobular artery. The vessels have been cut longitudinally. Note the absence of lymphatic channels around the intertubular capillaries. Hematoxylin and eosin,  $\times 274$ .

*C*, small lymphatic channels forming a network about an interlobular vein in the outer half of the cortex. Hematoxylin and eosin,  $\times 547$ .



which in the preparations are not accompanied by lymphatic vessels. The lymphatic channels are decidedly more plentiful in the cortex than in the medulla.

Two separate systems of channels are demonstrable, each of which can be shown to begin blindly by the abrupt appearance in serial sections of a lymphatic vessel which then can be traced as a single channel. Farther on such a channel anastomoses with others, which enlarge progressively. One system of lymphatic channels has its beginning as tiny blind-ending vessels which lie in close contact with Bowman's capsule (fig 1 *B*). These channels gradually enlarge and form nets around both the arterial and the venous vessels of the cortex, beginning near the terminal branching of both arteries and veins (no lymphatic vessels are demonstrable about the stellate veins), however, no lymphatic network is demonstrable in these sections around the afferent or the efferent arterioles of the glomeruli, and none penetrate through Bowman's capsule (fig 1 *C*). The latter may occasionally be partly surrounded by segments of nets which wind loosely about adjacent vessels (fig 3 *A*), these lymphatic arcs are part of the perivascular network, however, and are only incidentally related to Bowman's capsule. The intertubular capillaries, likewise, have no demonstrable network of lymphatic channels, lymphatic channels winding loosely about the arteries and veins do, however, frequently come in chance contact with a convoluted tubule (fig 1 *B*). The lymphatic nets about the cortical arteries and veins are prominent, those of the arteries are, on the average, somewhat larger and run a straighter course (fig 3 *B*). Especially noteworthy are the lymphatic vessels which lie in close proximity to the large thin-walled venous channels that are prominent particularly in the outer half of the cortex (fig 3 *C*). The anatomic connection of those venous "sinuses" is not clearly known, in the serial sections studied they appear to be parts of the interlobular veins, which in this region are exceptionally large and thin walled. Accompanying the interlobular vessels (fig 4 *A*), the lymphatic channels progress toward the hilus, winding around the arcuate vessels (fig 4 *B*) and interlobar arteries and veins, and, finally, leaving the kidney at the hilus to terminate in the nodes on either side of the aorta.

Another system of lymphatic channels begins blindly as a network beneath the mucosa of the papilla (fig 5 *A*), lymphatic channels from this region ascend in a more or less straight line, gradually increasing in size, running parallel to the small blood vessels of the medulla (fig 5 *B*) and emptying into the larger lymphatic channels which surround the arcuate arteries and veins. Because of the opposing directions, with respect to increase in size of the lumen, which characterize the two groups of lymphatic vessels described, they are considered to be separate systems.

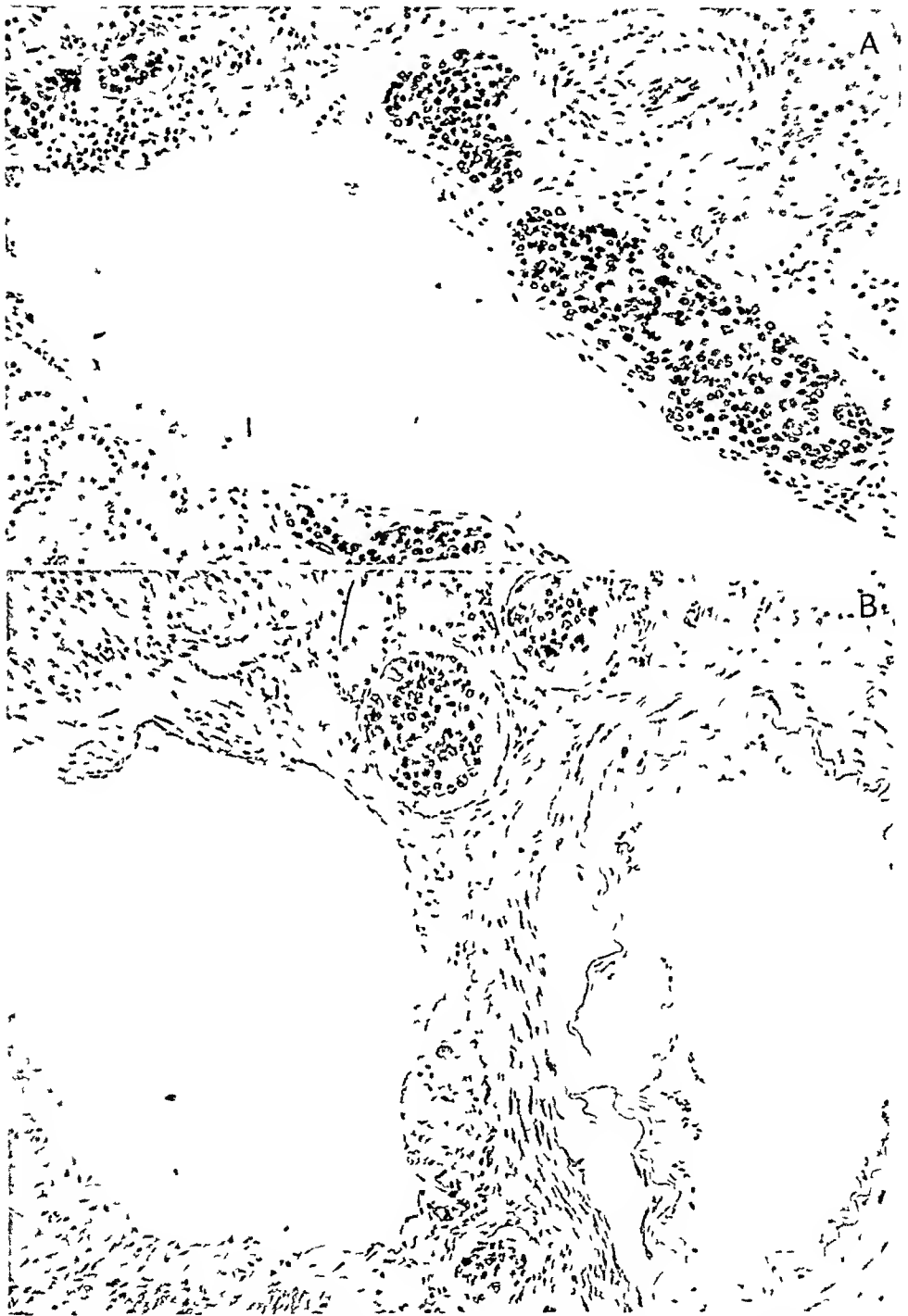


Fig 4—*A*, interlobular vein (near the corticomedullary junction) about which lymphatic channels are forming a rich network. Hematoxylin and eosin,  $\times 91$ .

*B*, large lymphatic trunks accompanying arcuate vessels. Hematoxylin and eosin,  $\times 108$ .

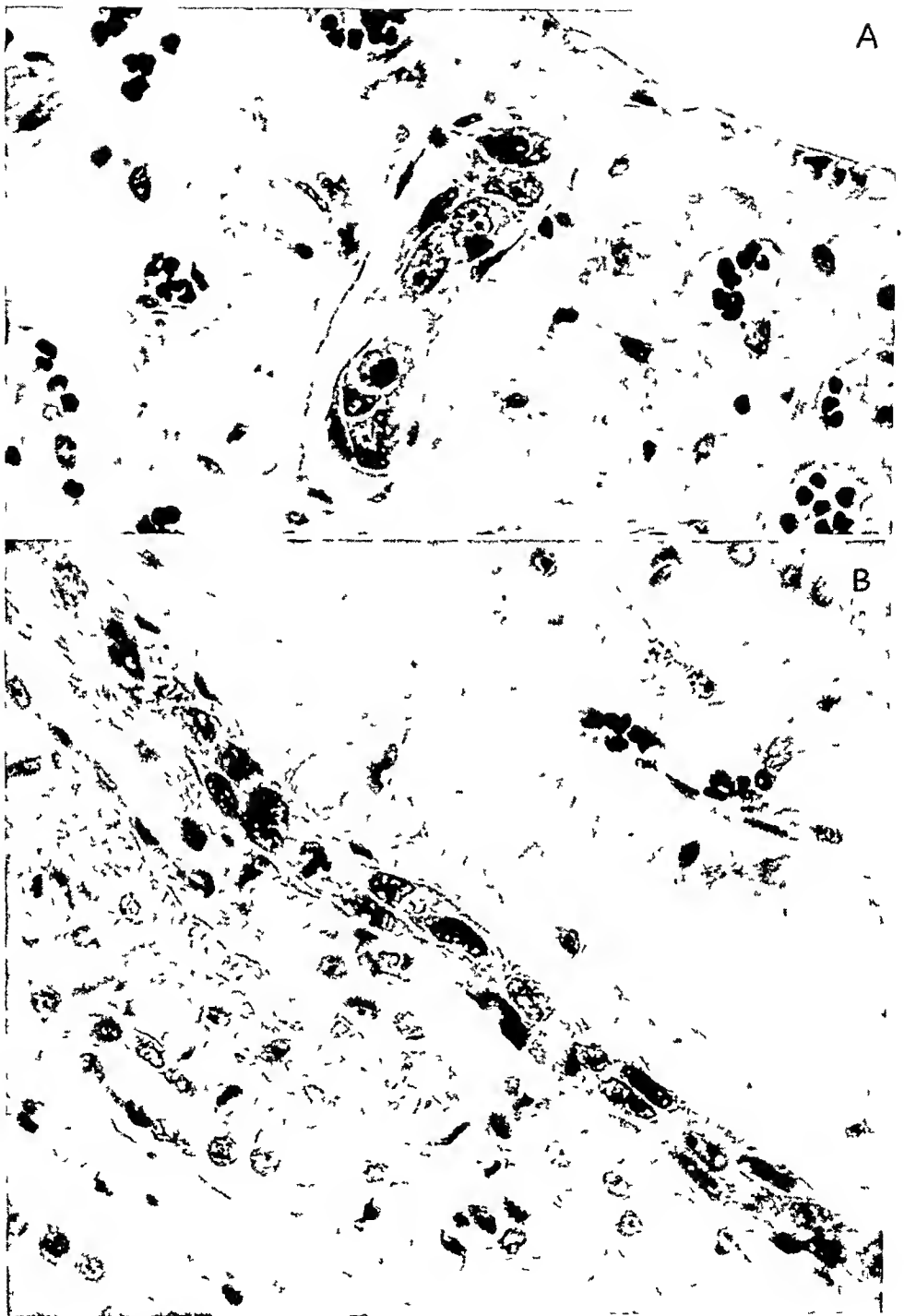


Fig 5—*A*, lymphatic vessel in a papilla. Serial sections show that this channel begins blindly beneath the mucosa, it passes through the medulla, running parallel to collecting tubules and blood vessels to terminate in the large lymphatic trunks about the arcuate vessels. Hematoxylin and eosin,  $\times 522.5$

*B*, medullary lymphatic vessel running parallel to tubules and blood vessels. The vessel starts as a blind end at the papilla and empties into the lymphatic trunks about the arcuate vessels. Hematoxylin and eosin,  $\times 497.5$

## COMMENT

Trueta and his co-workers<sup>9</sup> have recently offered convincing evidence for the existence of two hemic circulations, a greater and a lesser, in the kidney. Both take their origin from, or close to, the arcuate arteries. The greater circulatory pathway for blood consists of the interlobular arteries, afferent arterioles, glomerular capillaries, efferent arterioles, capillaries of the medullary rays, capillaries about the convoluted tubules, collecting veins and interlobular veins. The lesser circulatory pathway for blood consists of the afferent arterioles of the juxtamedullary glomeruli, these glomeruli themselves, their efferent arterioles and the vasa recta, arterial and venous, of the medulla. The studies here presented seem to indicate a double lymphatic system which parallels the venous component of this double hemic system. The greater lymphatic system, that of the cortex, accompanies the greater hemic system. The lesser lymphatic system, that of the medulla, appears to follow the course of the vasa recta of the lesser hemic system. The direction of flow in the lymphatic channels appears to be the same as that of the venous stream.

It has been shown by Schmidt and Hayman<sup>10</sup> that increased renal blood flow, in addition to augmenting urinary output, will increase the lymph flowing from the kidneys. It would thus seem that the renal lymphatic vessels may have an important regulatory function, preventing too much fluid from being excreted, and also preventing the accumulation of fluid in the kidney itself. The close relationship between the wide, thin-walled veins of the outer half of the cortex and the numerous fine lymphatic channels which form a network about them suggests the possibility that an exchange of fluid may take place in this region.

## SUMMARY

The lymphatic channels of the human kidney have been traced in a case in which permeating carcinoma cells made these channels easily visible.

It was found that the lymphatic channels begin blindly in two locations, namely, closely adjacent to the capsule of Bowman, and beneath the mucosa of the papilla. From these origins two networks arise which accompany the arterial and the venous vessels of the kidney. The network arising in the medulla drains upward, toward the arcuate vessels, whereas that beginning near Bowman's capsule drains in the opposite direction. The two become confluent about the arcuate vessels.

9 Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., and Prichard, M. M. L. *Studies of the Renal Circulation*, Springfield, Ill., Charles C Thomas Publisher, 1947.

10 Schmidt, C. F., and Hayman, J. M., Jr. *Am J Physiol* **91** 157, 1929.

No lymphatic channels are demonstrable in the glomeruli, about the afferent or the efferent arterioles, or about the intertubular capillaries. If such networks exist, their caliber is too small to permit carcinoma cells to permeate them.

Especially noteworthy is the close association of the lymphatic channels and the large thin-walled veins of the outer half of the renal cortex. It is believed that these veins are expansions of the interlobular veins and their tributaries and that the closely approximated lymphatic and venous channels may form a regulatory system for fluid exchange.

# Case Reports

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## MICROCYSTIC (THYROID-LIKE) KIDNEY

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WICHITA, KAN

**T**HYROID-LIKE dilatation of renal tubules is largely regarded as a result of pyelonephritis<sup>1</sup>. This opinion is so general that few original articles have been written on the subject.

A case is now reported, first, because no description of similar extensive dilatation of tubules could be found in the literature and, second, because this case raises serious doubt whether thyroid-like kidneys are always due to pyelonephritis.

### REPORT OF A CASE

A 22 year old Negro woman was admitted to the hospital on Nov 29, 1947. She complained of stiffness and soreness of all joints, a condition which had been present for the past year. The patient had been seen by a physician in Oklahoma during the course of her illness, but information relating to the diagnosis and the medication was not available. For the past three weeks the patient had observed that her arthritic symptoms were becoming more severe and that marked lassitude had developed.

Examination revealed an emaciated, drowsy Negro woman. There was fusiform swelling of the interphalangeal proximal joints with stiffness of elbows and knees. The pulse rate was 96, the temperature, 97 F.

A high white cell count suggested infection, and 50,000 units of penicillin was given every three hours. The patient was drowsy and semicomatose throughout her hospitalization. She underwent minor convulsions and died in apparent uremic coma two days after admission.

The Wassermann and Kline tests of the blood showed 4 plus reactions. The specific gravity of the urine was 1.010, the urine contained albumin (2 plus), occasional red blood cells and clumps of white blood cells. The hemoglobin was 6.1 Gm per hundred cubic centimeters. The white cell count was 20,000, and the differential count showed segmented forms (67 per cent), band forms (22 per cent) and lymphocytes (10 per cent).

Postmortem inspection revealed a well developed but emaciated Negro woman. There was slight swelling of the proximal joints of the fingers. No edema, jaundice or lesions of the skin were visible. The pericardial sac contained 8 cc of sero-fibrinous fluid. The right auricle and ventricle were flabby. There was hypertrophy of the left ventricle. Each kidney weighed 160 Gm and measured 11.5 by 6 by 4 cm. The capsule stripped with ease. The surface was pale grayish brown and peppered with minute rounded opaque areas 1 mm in diameter. No petechiae were visible. There was indistinct shallow lobulation with several shallow scars. Section revealed a pale grayish brown cortex with honeycombed structure. The orderly arrange-

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From the Department of Pathology, St Francis Hospital

1 (a) Boyd, W. Canad M A J **47** 128, 1942 (b) Weiss, S, and Parker, F, Jr. Medicine **18** 221, 1939 (c) Mallory, G K, Crane, A R, and Edwards, J E. Arch Path **30** 330, 1940

ment of the renal pyramids with the apexes converging toward the renal sinus was slightly distorted, for in one area the renal columns of Bertini were prominent, overshadowing the adjoining pyramid, while in another pyramid the apex was directed into the calix laterally (fig 1) Calices, pelves and ureters showed no dilatation or gross abnormalities

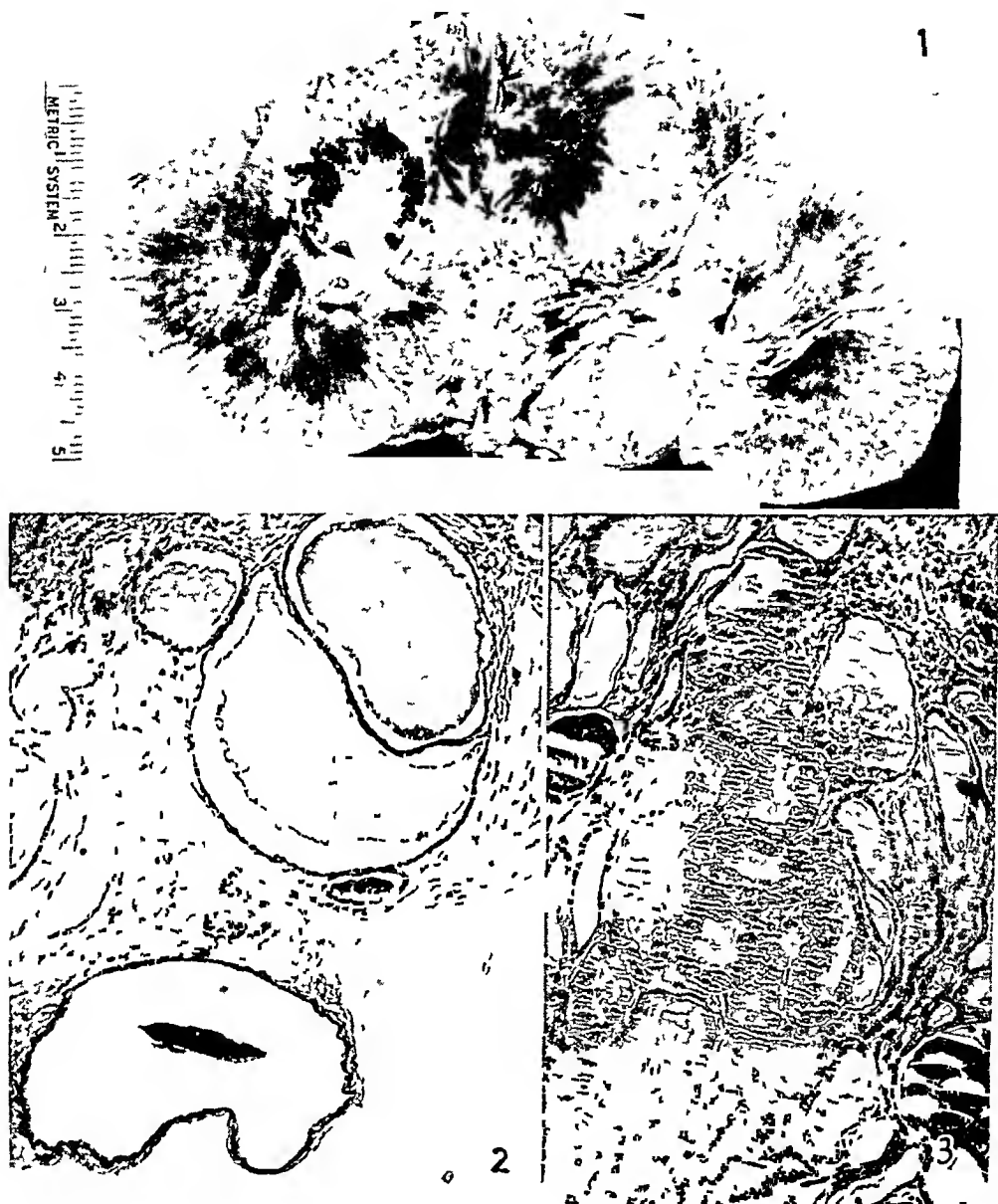


Fig 1—Longitudinal section of the microcystic kidney. Note macroscopic cysts and slight distortion of structure.

Fig 2—Dilated tubules protruding into the calix,  $\times 100$ . There is little inflammatory reaction in the papillae.

Fig 3—Degenerative changes in tubules,  $\times 100$ .

Microscopically, the majority of the glomeruli revealed a dilated capsule filled with a light pink homogeneous material. The glomeruli revealed varying changes. Many were set off by pericapsular fibrosis. The ones surrounded by dense fibrous

tissue were shrunken, and different stages of obliteration with early hyalinization were apparent. No epithelial crests were visible. Most of the glomeruli showed tufts that varied in size, some being large and cellular, others revealing only a small cellular mass at the periphery as though compressed by the surrounding fluid.

Nearly all the tubules appeared large and contained a pink-staining "colloid" of different densities. The majority of the tubules of both cortex and medulla showed enormous cystlike dilatations lined by a single layer of flattened epithelium. Many dilated tubules could be followed for some length without interruption. Others showed the wide cystlike spaces separated by thin septums or partial septums, the cysts lying adjacent to each other in a linear fashion and extending for great lengths in the cortex, medulla and papillae. Many cross sections through the cortex, medulla and papillae showed similar cystlike structures. Tubules that did not present the enormous cystlike dilatations were generally larger than normal and had a well defined epithelium, these also contained the "colloid," deep red-stained material. Small, apparently normal tubules were found in the sections. Occasional polymorphonuclear leukocytes were noted in the tubules. Cloudy swelling and varying stages of degeneration of the tubules, particularly the proximal convoluted ones, was noted.

The medulla revealed an increased amount of loose vascular stroma with scattered areas of round cell infiltration and a sparse scattering of leukocytes. The connective tissue and round cell infiltration of the cortex was more diffuse, dense and prominent. The epithelium of the calices and pelves was of the transitional type, with no thickening or metaplasia. The blood vessels showed no intimal or medial thickening.

#### COMMENT

Doubt as to whether inflammation was the cause of this unusual microcystic kidney arose from the observation that there was no chronic papillitis or fibrous barrier in the medulla. Staemmler and Dophiede,<sup>2</sup> in their description of chronic contracted pyelonephritic kidneys, stated that there is slowly progressive destruction of the cortex, terminating in a thyroid-like tissue, due largely to the productive medullary inflammation, which in turn causes fibrosis of the pyramids. This fibrosis forms a barrier near the corticomedullary junction and produces obstruction of the tubules with resultant thyroid-like cystic dilatation.

While in this case inflammation was present in both cortex and medulla, it apparently had not interfered with the continuity of the tubules and the pelvis. Figure 2 shows that small cysts may protrude into the pelvis with no fibrous tissue separating pelvic epithelium and cysts. These kidneys showed definite inflammatory and degenerative changes, but the changes were confined largely to the cortex. Tubular degeneration with swelling, oxyphilic epithelial staining and occasional loss of nuclear stain are seen in figure 3. Round cell infiltration, increase of fibrous tissue and all stages of degeneration of glomeruli appeared. Figure 4 reveals one of the characteristic glomeruli with surrounding inflammatory changes.

Polymorphonuclear leukocytes in small numbers were present in a few of the collecting tubules. However, since most of the tubules

2 Staemmler, M., and Dophiede, W. *Virchows Arch f path Anat* 277 713, 1930



contained colloid-like material without leukocytes, Malloy's<sup>1c</sup> view that the material is derived from imprisoned disintegrating leukocytes is questioned

Varying densities of the albuminous material were observed in nearly all the tubules and most of the glomeruli. Thus, the theory of Staemmler and Dophiede<sup>2</sup> that the material is a product of atrophic tubular epithelium does not explain the findings in this case

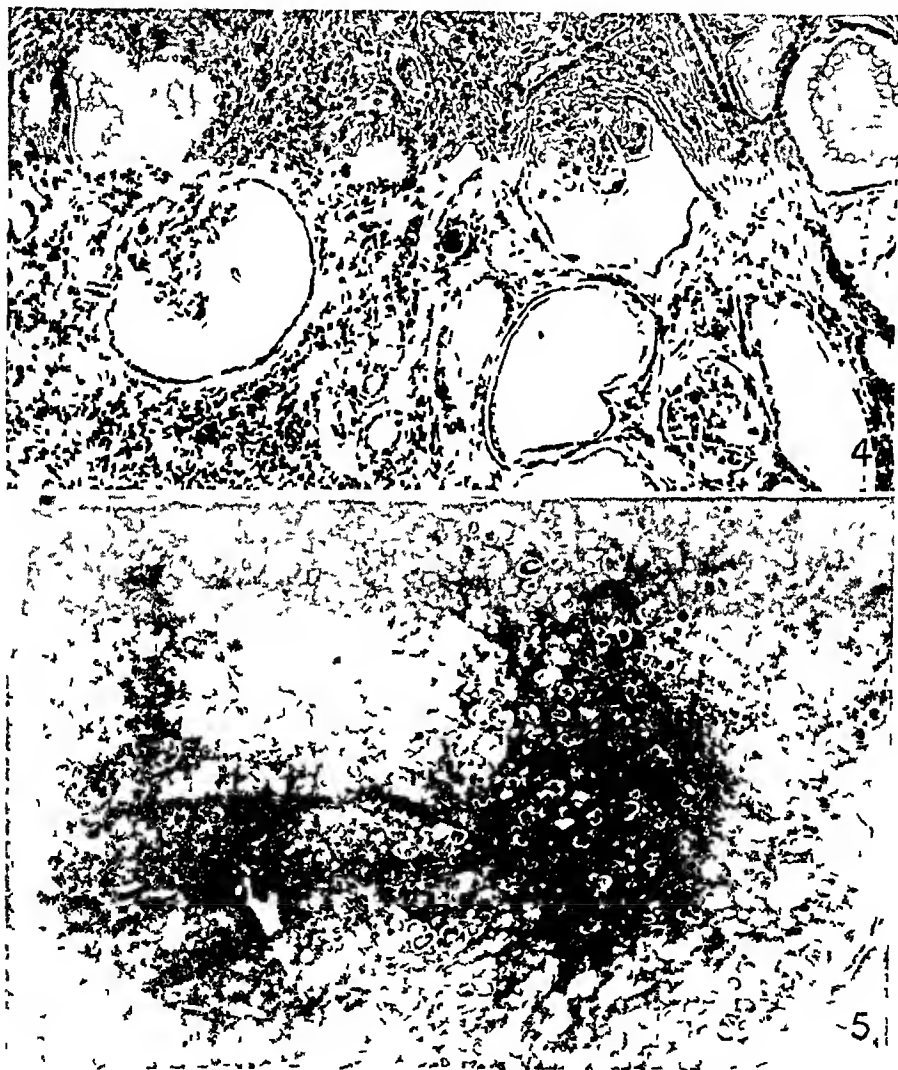


Fig 4—Cortex showing periglomerular fibrosis with shrunken tufts and "colloid" material in the glomerulus,  $\times 100$

Fig 5—Cysts filled with "colloid" in cortex and medulla, approximately  $\times 4$

Many of the cystic spaces, ranging from 50 microns to 1 mm in diameter, could be followed in a linear fashion for some length in both cortex and medulla. They were separated only by thin septums or partial septums without the cortical localization of the pyelonephritic contracted kidney but rather the diffuse distribution seen in polycystic kidneys (fig 5)

For these reasons I believe that this case should be regarded as one of a form of congenital cystic kidney

The reconstructive works of McKenna and Kampmeier,<sup>3</sup> Norris and Herman<sup>4</sup> and Lambert<sup>5</sup> largely disprove that cystic kidneys develop because of the failure of the two anlagen to unite, incomplete development of the tubules or fibrotic obstruction of tubules

Kampmeier<sup>6</sup> and later McKenna and Kampmeier<sup>3</sup> demonstrated that the first three or four generations of uriniferous tubules are not permanent but break away from their respective collecting ducts and undergo cystic degeneration during normal development of the kidney. They suggested that persistence and growth of these fetal cystic structures offer an explanation of the origin of cystic kidneys

According to Norris and Herman,<sup>4</sup> there is normal development of the kidneys for a long period of fetal life, and focal cystic dilatation of uriniferous tubules and collecting tubules occurs after differentiation and union of the anlagen, thus there is offered an explanation for the presence of cysts arising in the collecting tubules as well as in the uriniferous elements

The inflammatory changes which finally led to renal failure in this case are best regarded as secondary interstitial nephritis, a not uncommon complication of congenital cystic kidney

#### SUMMARY

A case of microcystic, thyroid-like kidney is reported. The patient was a 22 year old Negro woman. The cut surface resembled diffuse colloid goiter, and cystic tubules were found in the cortex as well as in the medulla

Both kidneys were of normal size and did not present the usual evidence of chronic pyelonephritis, which is generally regarded as the cause of thyroid-like kidneys. From the study of this case it is concluded that not all thyroid-like kidneys can be attributed to blockage of the collecting tubules following chronic pyelonephritis, but that some of these kidneys are best interpreted as a congenital malformation of renal tubules

3 McKenna, C M, and Kampmeier, O F. *J Urol* **32** 37, 1934

4 Norris, R F, and Herman, L. *J Urol* **46** 147, 1941

5 Lambert, P P. *Arch Path* **44** 34, 1947

6 Kampmeier, O F. *Surg, Gynec & Obst* **36** 208, 1923

## RUPTURE OF THE CORONARY SINUS FOLLOWING MYOCARDIAL INFARCTION

DAVID B HINSHAW, M D  
AND

ALBERT F BROWN, M D  
LOS ANGELES

REFERENCES to the coronary sinus or great vein of the heart are scarce in medical literature. Various investigations have been carried out in regard to the physiologic function of this structure. It is generally stated that about 60 per cent of the venous blood return from the heart muscle is by way of the coronary sinus—the remaining venous return being by way of the thebesian vessels and through direct communications between the coronary arteries and the ventricular cavities. The coronary sinus is not subject to pathologic changes to any appreciable extent. External trauma would perhaps comprise the major source of its pathologic changes.

The case reported in the following pages is one in which a myocardial infarction due to coronary thrombosis resulted in a rupture of the coronary sinus. No references to any previous instances of rupture of the coronary sinus could be found in the *Index Catalogue of the Surgeon General's Office* and the *Quarterly Cumulative Index Medicus*.

### REPORT OF A CASE

The records of this case have been used with the permission of Dr B P Mundall.

V G, a 72 year old white Caucasian man of medium height and weight, was admitted to the Glendale Sanitarium and Hospital in February 1943. He had had recurrent attacks of pyelonephritis for three years, also, personality changes had been noted by his family for several months. His chief complaint on being admitted was severe thoracic pain radiating to the left arm and the upper part of the back. Opiates were required to relieve this pain.

The pulse rate was 100 per minute, the respiratory rate 28 per minute and the blood pressure 140 mm of mercury systolic and 90 mm diastolic. Heart, lungs and abdomen were not unusual. No edema was present. The reflexes were all somewhat hyperactive but equal bilaterally, both Babinski reactions were equivocal. The prostate gland was firm and moderately enlarged, no rectal masses were felt.

The urine was cloudy and on microscopic examination contained moderate numbers of pus cells and red blood cells, no sugar or albumin was present. The red blood cell count was 4,800,000, the hemoglobin, 17 Gm, the white blood cell count was 8,900, 89 per cent of which were mature neutrophils. The nonprotein nitrogen of the blood amounted to 124 mg per hundred cubic centimeters.

The patient became progressively worse. The thoracic pain continued, and his temperature varied between 99 and 101 F. He became irrational and died thirteen days after admittance.

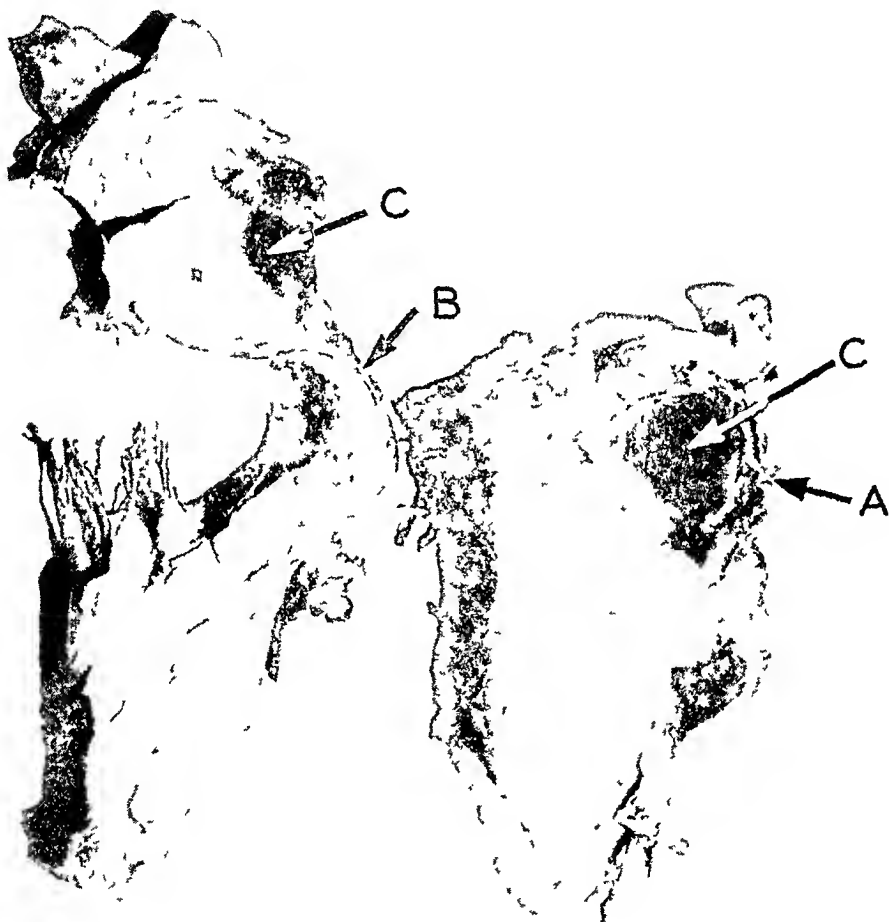
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From the Departments of Pathology and Medicine, Glendale Sanitarium and Hospital and College of Medical Evangelists

*Autopsy*—The body was that of a well developed, moderately well nourished white man aged 72 years. No external findings of importance were to be noted.

Cross sections of the brain had areas of moderately old necrosis with partial cystic degeneration in the corpus striatum of each side. Further sections of the brain showed a 6 mm hemorrhagic discoloration in the cortex of the right parietal occipital region, apparently due to recent infarction.

The pleural cavities were without significant change. The lungs were moderately heavy and on the cut surface showed edema and congestion. The pericardial sac



Cross sections of lateral and posterior portions of the left ventricle showing the coronary sinus distended with blood and communicating with an area of hemorrhagic infiltration of the epicardium. A indicates the area of rupture of the terminal portion of the coronary sinus, B, a cardiac aneurysm, C, the coronary sinus.

was distended with clotted blood. Some of this clot was soft and red, apparently fresh. Other portions of the clot showed discoloration and suggestive platelet lines, indicating older hemorrhage. This portion of the clot was strongly adherent to the epicardium.

The heart was approximately normal in size. The ventricular cavities were not dilated. The wall of the left ventricle was 12 cm thick except in the upper lateral portion of the left ventricle where the wall was scarred, thinned to 5 mm and pouched outward. No evidence of rupture was found in this area, but the scar tissue showed hemorrhage and discoloration, suggestive of recurrent necrosis.

There was an aneurysmal sac about 2 cm in diameter and about 1 cm deep. The remainder of the lateral wall of the left ventricle showed a large area of moderately recent infarction in which the myocardium was discolored yellow-gray and had a somewhat gelatinous texture. No point of rupture was found in this area. The epicardium was especially involved in the hemorrhagic process along the lateral left atrioventricular margin. The coronary sinus in this region appeared to be greatly distended with clotted blood, and there was a suggestion of a defect in its wall in an area continuous with the region of intraepicardial hemorrhage. The wall of the coronary sinus at this point was also in close relation to the old aneurysmal scar and area of necrosis. A thin layer of mural thrombus was attached to the inner aspect of the aneurysm. The coronary arteries were severely atherosclerotic, and the left circumflex branch was occluded by a pink-red thrombus for a distance of about 1 cm beginning about 1.5 cm from its origin.

No significant changes were found in the peritoneum, the gastrointestinal tract or the abdominal viscera except for slight chronic passive congestion.

Microscopically the brain revealed areas of moderately old softening and disintegration of tissue.

Sections of the kidneys were not significant except for a small amount of edema of the renal cortex.

Sections of the heart warrant a detailed description. A section from the midportion of the lateral wall of the left ventricle showed a layer of organizing blood clot covering the epicardial surface. The organizing process had progressed to a considerable depth in the clot. The underlying myocardium showed complete recent necrosis which involved all but narrow layers near the epicardium and the endocardium. Large areas of undissolved myocardial fibers were still present in the infarct, but there was much granulation tissue and early fibrosis, especially near the blood vessels. One area showed older fibrous tissue, apparently that of a previous scar. Sections taken from the base of the left ventricle through the coronary sinus and the upper border of the old cardiac aneurysm showed the coronary sinus to be filled with recent blood clot. Portions of the clot which lay near the periphery exhibited platelet lamination and some degree of organization. The wall of the coronary sinus appeared involved in the necrosis of the adjacent myocardium and had apparently ruptured, allowing blood to dissect into the epicardial tissues. A peculiar hyperplastic reaction was found in the mesothelium of the epicardium, with the formation of multinucleated cells in some places. A portion of the left circumflex coronary artery, which was included in one of the sections, showed severe atherosclerosis and thrombotic occlusion.

*Anatomic Diagnoses*—Arteriosclerotic heart disease, coronary thrombosis, old myocardial infarction with cardiac aneurysm, recent myocardial infarction, rupture of the coronary sinus due to its having been included in the area of infarction, organizing hemopericardium, mural thrombus of the left ventricle, cerebral softening, possibly embolic.

#### SUMMARY

A case of rupture of the coronary sinus of the heart associated with myocardial infarction is presented. As nearly as can be determined, no similar case has as yet been described in medical literature.

# Laboratory Methods and Technical Notes

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## RETICULUM SILVER IMPREGNATION FOR OLD FORMALDEHYDE-FIXED TISSUE

ELENA DE GALANTHA  
HOUSTON, TEXAS

THE usual methods employed for the staining of reticulum are not applicable to tissue that has been kept in formaldehyde solution U S P for long periods. The method to be described has given good results with tissue kept in formaldehyde solution as long as ten to twenty years.

### METHOD

- 1 Cut in half-centimeter square blocks
- 2 Rinse free of formaldehyde solution in tap water (two hours)
- 3 Put blocks in 3 per cent strong ammonia solution U S P for twenty-four hours
- 4 Wrap each block in a piece of gauze and put the wrapped blocks in a dish under slowly running tap water for twenty-four hours
- 5 Dehydrate in 75 per cent alcohol for one hour
- 6 Dehydrate in 95 per cent alcohol for one hour
- 7 Place in acetone for three hours
- 8 Clear in xylene for one hour
- 9 Impregnate with soft paraffin for one hour
- 10 Embed
- 11 Cut sections at 5 to 6 microns
- 12 Dry in a 37 C oven overnight
- 13 Deparaffinize in xylene (two changes) and 95 per cent alcohol, then pass through chloral hydrate (saturated solution) and into distilled water
- 14 Place slides in coplin jars with 5 per cent silver nitrate for twenty-four hours in a 37 C oven
- 15 Wash quickly in distilled water
- 16 Place slides in silver ammonium oxide for five to ten minutes (Silver ammonium oxide is prepared as follows: to 100 cc of 5 per cent silver nitrate add 5 drops of 40 per cent sodium hydroxide. Clear with strong ammonia solution U S P, drop by drop, and shake until clear.)
- 17 Wash slides in distilled water carefully and place them in a solution made of 1 part of 40 per cent formaldehyde solution to 9 parts of tap water, for ten minutes
- 18 Wash quickly in distilled water

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From the Department of Pathology, M D Anderson Hospital, University of Texas

19 Carefully reduce the excess of precipitate in 1 per cent gold chloride (brown) with 2 drops of acetic acid

20 Wash in water quickly, clear in 5 per cent sodium hyposulphite for one minute and rinse quickly in distilled water

21 Clear in 50, 70, 80 and 95 per cent alcohol and xylene Apply cover slip with Canada balsam

NOTE This method is good for fresh formaldehyde-fixed tissue, but it does not need the prolonged process Avoid nos 2, 3 and 4 In no 14 reduce the time to five hours

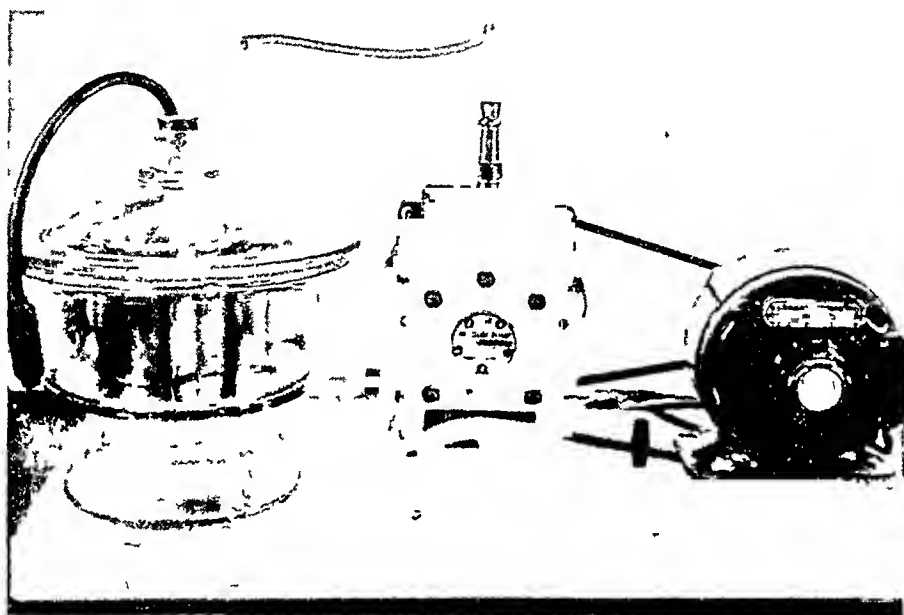
#### RESULTS

Reticulum is stained intense black, and the background is almost colorless

# TECHNIC OF VACUUM PARAFFIN INFILTRATION OF TISSUE ADAPTED TO THE USE OF THE TECHNICON

CRICHTON McNEIL, M D  
SALT LAKE CITY

AFTER fixation and dehydration, tissues must be exposed to paraffin for two to five hours, depending on the size of the section, to obtain satisfactory embedding. Pathologic laboratories have made use of vacuum to obtain better paraffin infiltration,<sup>1</sup> but no reports are available and no attempt to adapt this technic to the use of the automatic "technicon" is known.



The vacuum type desiccator jar at the left holds the "technicon" paraffin container and basket. Electrical connection is maintained through a rubber stopper, which also carries the exhaust tube directly to the vacuum pump.

## PROCEDURE

Tissues are placed in the "technicon" basket at jar 1, which contains a 4 per cent formaldehyde solution in 70 per cent ethyl alcohol, where they remain until 9 p.m. Thereafter until 7 a.m. they are passed at hourly intervals through the usual graded alcohols, dioxane, dioxane and paraffin, and finally into paraffin. At 8 a.m. the entire heated paraffin jar with the tissue basket is detached from the rotator and placed in the 200 mm diameter Scheibler desiccator jar, which has a two hole rubber stopper in the lid (figure). One outlet is attached to the vacuum

From the Laboratories of Holy Cross Hospital and the Department of Pathology, University of Utah School of Medicine

1 Landau, E. Bull d'histol appliq a la physiol 16 13, 1939



pump<sup>2</sup>, the other, containing the wire, is plugged into a regular electrical outlet. Vacuum is quickly obtained and the motor turned off so that too great a vacuum and foaming are avoided. This negative pressure corresponds to a mercury column of 294 mm.

Experience has shown that in this vacuum a one-half hour exposure of tissues is adequate for complete impregnation. The tissues are blocked as usual.

The following advantages have been observed. Tissues cut with greater ease, and flaws are eliminated, thinner sections can be obtained, because of better impregnation, in the "technicon" there is more time for dehydration, tissues are not "cooked" in paraffin.

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<sup>2</sup> The W. M. Welch Manufacturing Company Duo Vacuum pump model 1400 B is used.

## Books Received

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ZINSSER'S TEXTBOOK OF BACTERIOLOGY THE APPLICATION OF BACTERIOLOGY AND IMMUNOLOGY TO THE DIAGNOSIS, SPECIFIC THERAPY AND PREVENTION OF INFECTIOUS DISEASES FOR STUDENTS AND PRACTITIONERS OF MEDICINE AND PUBLIC HEALTH Revised by David T Smith, M D, professor of bacteriology and associate professor of medicine, Duke University School of Medicine, Donald S Martin, M D, M P H, professor of preventive medicine and public health and associate professor of bacteriology, Duke University School of Medicine, Norman F Conant, Ph D, professor of mycology and associate professor of bacteriology, Duke University School of Medicine, Joseph W Beard, M D, professor of surgery in charge of experimental surgery, Duke University School of Medicine, Grant Taylor, M D, associate professor of bacteriology and associate professor of pediatrics, Duke University School of Medicine, Henry I Kohn, Ph D, M D, surgeon, U S Public Health Service, assistant professor of physiology and pharmacology (on leave), Duke University School of Medicine, Mary A Poston, M A, instructor in bacteriology, Duke University School of Medicine Ninth edition Pp 992, with 251 illustrations Price \$10 New York Appleton-Century-Crofts, 1948

The inheritance of the responsibility of authorship of an established textbook is a mixed blessing to the new authors Patterns good and bad have been set, and precedent discourages their alteration Changes must be gradual, if due respect is to be shown for the judgment of past authors This consideration exercises more influence in some instances than in others So it is with considerable trepidation that this reviewer approaches the task of reviewing the current edition of the "Textbook of Bacteriology," first edited by Hiss, added to by Zinsser, then by Bayne-Jones, and currently by Smith and Martin

In general the new edition retains the arrangement of the previous edition New material has been introduced dealing with antibiotics and with pleuropneumonia-like organisms In connection with the discussion of specific infections, new emphasis is placed on the public health significance of diseases Revisions in text material have been made to include results of current investigations By and large the material presented throughout the book is accurate, readable from a medical student's point of view, and valuable to any one interested in the subject of infectious diseases of man Like most medical textbooks, it is too long Failure to give differential emphasis to first things, and to save words by placing minutiae in appropriate tables, or eliminating them altogether, leads to criticism, although it certainly follows the precedent of current writing of medical textbooks Many lines are wasted in describing isolated recoveries of pathogens from various animals, biologic properties which might be of interest at some future time, or morphologic or cultural variations not correlated with clinical problems This is the type of information which the reviewer would prefer to see in tabular form, subject to reference but not impeding the reading of the student who seeks knowledge of the principles of medical bacteriology The physical sciences have long since given up describing details in the text and instead relegate such information to appropriate tables or charts A textbook must be read by students One wonders, therefore, why the authors incorporate six pages of detail on the antigenic configuration of the salmonella in the body of the book Could this material not better be placed in an appendix, so as not to distract the student from the important job at hand?

The selection of references is certainly a difficult problem. Obviously it is not possible to cite all the books relating to a given topic, nevertheless, it is important that careful consideration be given to avoiding provincialism. There are evidences of this error in the new edition, even as in practically any textbook. Certain English writers seem to have a happier faculty for selecting more universal reference lists. Since textbooks are used—or at least it is the hope of the publisher that they will be—in schools the country over, this criticism assumes a practical nature. Since a topic can be only incompletely documented with references, in the reviewer's opinion authors of textbooks should be careful to make sure that there is a fair geographic distribution of emphasis, provided this is indicated by the merit of the works.

The typography of the new edition is excellent, and monotony is avoided through the frequent introduction of significant charts and pictures. Many new illustrations have been added. Unfortunately, not all the pictures and charts are well reproduced, and a few appear to have been selected more for their uniqueness than for their value in illustrating a principle. The selecting of pictures for a textbook demands as much discrimination as that of the items to be included in the text. Dramatic or unusual pictures may interest the novice or the expert but they confuse the student. There is much good taste shown by the authors of this textbook in following the principle indicated. This holds particularly for the section dealing with the higher bacterial forms. On the other hand, some yielding to temptation is evident in the use of unusual pictures from the field of virology which may not seem worth while to all readers.

The importance of presenting general principles of medical bacteriology and immunology is acknowledged in varying degrees by different writers of textbooks. In general there is less tendency among bacteriologists than among pathologists to develop the general aspects of their topic before going into details of diseases. Since the principles are of prime importance, it is regretted that the authors have not given more consideration to the fundamentals of infection, resistance, immunology and the general physiology of micro-organisms. The pattern of the earlier editions possibly influenced the authors in their emphasis, yet another approach might have served equally well the purpose of developing a knowledge of principles on the part of users of the book. The problem of the degrees to which bacteria are destroyed by chemicals and antibiotics *in vitro* and *in vivo* is inadequately presented. Bacteriostatic action and bactericidal action of disinfectants are not well differentiated. No reference is made to some of the more recent experiments in determining the effectiveness of disinfectants by methods designed to differentiate between bactericidal and bacteriostatic action.

There are advantages and disadvantages in adding a section on practical methods to a textbook on bacteriology. If such a section is added, then it should be fairly complete. The reviewer believes that this section of the textbook in question is not as carefully prepared as it might have been, revealing a fair number of sins of omission and commission.

The reviewer, having exhausted the details of all that is or could be wrong with this book, now would like to indicate in general terms that this ninth edition of Zinsser's text, edited by Smith and Martin, can easily be considered a first class work among the available American textbooks in medical bacteriology. It is the reviewer's opinion that a great many of his criticisms could equally be leveled against many other textbooks of bacteriology in the field today. Certainly no medical student who is assigned this book as a text will fail to learn his subject through any failure of the book studied. Also, any medical graduate may find a great deal of valuable information in this book, readily available and presented in a readable manner. The evidence of the improvement of this text over the eighth edition is obvious.

## ASPHYXIA NEONATORUM AND THE VERNIX MEMBRANE

FRED DICK Jr, M D  
AND  
EDGAR R PUND, M D  
AUGUSTA, GA

A SIGNIFICANT number of asphyxial neonatal deaths are believed to be caused by vernix caseosa plugging the bronchioles and lining the alveolar ducts and walls. The presence of a vernix membrane may represent only a more serious manifestation of the aspiration of amniotic contents, but since no proof exists as to its causation, it may be discussed as a distinct entity. Although this condition has been frequently recognized by many investigators who have been concerned with infant mortality and experimental physiology, too little emphasis has been placed on it as a primary cause of death in liveborn infants.

Observers reporting on the vernix membrane in current literature and textbooks persist in discussing it in connection with pneumonia of the newborn and the stillborn. The membrane was first described by Johnson and Meyer<sup>1</sup> in 1925. During a study of pneumonia they encountered several newborn infants with a hyaline membrane coating the walls of the smaller respiratory passages. This membrane stained intensely with eosin, contained many fat droplets and gave a negative result when stained for fibrin. They believed that it was derived from dissolution of the epidermal cells and fat of vernix caseosa, which was converted into a viscous layer. The presence of the membrane was associated with pneumonia in some but not in all instances. The membrane was never observed in stillborn infants. Therefore it was believed that the membrane was formed only after respiration had been established and that intrauterine aspiration of amniotic fluid was a necessary antecedent. Johnson and Meyer therefore concluded that intrauterine aspiration of fluid must follow respiration established in utero as a result of some factor producing anoxia. Farber and Sweet<sup>2</sup> also expressed the belief that the intrapulmonary presence of large amounts of amniotic contents was an indication of intrauterine aspiration following intrauterine anoxia. In a presentation of 178 cases in which amniotic contents had been found in the lung, they

From the Department of Pathology, University of Georgia School of Medicine.

1 Johnson, W C, and Meyer J R. *Am J Obst & Gynec* 9 151, 1925

2 Farber, S, and Sweet, L K. *Am J Dis Child* 42 1372, 1931

reported three neonatal deaths associated with vernix membrane in infants delivered by postmortem cesarian section Russ and Strong found that the infant mortality rate following cesarian section was 9 to 10 per cent when inadequate intratracheal aspiration was performed and less than 2 per cent when aspiration was done Infants not treated with proper aspiration lived from four to forty-seven hours and died with symptoms of obstruction of the respiratory passages In each one a "pseudo-membrane" was observed in the alveoli of the lungs, and they considered that this condition was a true aspiration pneumonia Helwig,<sup>4</sup> in a report of 66 cases of pneumonia, mentioned finding a vernix membrane in the lungs in 2 Rosenthal<sup>5</sup> attributed the membrane to degenerative changes in the epithelial lining of the respiratory passages due to intrauterine anoxemia, a "desquamative anaeriosis" Benner<sup>6</sup> said that in some cases in which there has been aspiration of large amounts of amniotic contents "there is evidence that respiration has been attempted, as the vernix appears to have been forced against the alveolar walls, where it lies as a membrane and forms a barrier to gaseous exchange This membrane must therefore be recognized as a cause of asphyxia and early death of the infant" Macgregor<sup>7</sup> found 11 infants with hyaline membrane in a series of 541 consecutive necropsies on the newborn and expressed the belief that it resulted from aspiration of amniotic contents in utero following intrauterine anoxemia In all cases pulmonary atelectasis was more severe than usual Ample evidence of severe anoxia indicated that the infants had been asphyxiated at birth She, too, stated that the vernix membrane often occurred without inflammatory reaction, which indicated that pneumonia was merely a complication and not the cause of the presence of the membrane Labate<sup>8</sup> found the hyaline membrane in 2 per cent of fetal or neonatal deaths attributed to lesions of the lungs He stated that from the point of view of etiology the condition has not been explained but that it may represent a late effect of intrauterine aspiration of amniotic fluid The fluid may be absorbed and the solid elements converted into a hyalinized gummy substance Potter<sup>9</sup> not only mentioned the hyaline membrane as a cause of neonatal death but thought that its presence should be suspected on a study of the clinical course of the newborn Schenken<sup>10</sup> also wrote of this condition in his report of a series of deaths due to aspiration of amniotic contents

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3 Russ, J D, and Strong, R A *Am J Obst & Gynec* **51** 643, 1946

4 Helwig, F C *Am J Obst & Gynec* **26** 849, 1933

5 Rosenthal, M *J Pediat* **6** 71, 1935

6 Benner, M C *Arch Path* **29** 455, 1940

7 Macgregor, A R *Arch Dis Childhood* **14** 323, 1939

8 Labate, J S *Am J Obst & Gynec* **54** 188, 1947

9 Potter, E L *Am J Clin Path* **17** 524, 1947

10 Schenken, J R *Nebraska M J* **32** 362, 1947

## DESCRIPTION OF PATIENT AND MEMBRANE

Some of the infants who live for a period of a few hours to four days after birth exhibit fairly characteristic clinical signs of disease of the respiratory system prior to death. They may breathe normally or be slow to breathe at birth but eventually there develops an increasing struggle for breath. Dyspnea and cyanosis become manifest in spite of oxygen-carbon dioxide insufflation, and costal retraction may occasionally be present. At necropsy the changes are frequently limited to the lungs. The lungs of these infants are liver-like in consistence and sink when placed in water. Only a slight amount of aeration may be observed, along the anterior margins. Microscopically, a hyaline membrane is seen which stains with eosin. The membrane is plastered against the walls of opened alveoli, alveolar ducts and an occasional respiratory bronchiole. With sudan IV the membrane is stained red, both diffusely and in many small droplets. Toward the luminal side of the membrane one can occasionally see a basophilic stringy substance which gives a negative reaction for mucin. A variable number of epidermal cells may be found in this location. Vernix caseosa of exceptionally high lipid content and containing many epidermal cells is seen plugging the terminal bronchioles where these join the alveolar ducts. The alveolar ducts and alveoli which are lined with the membrane remain open. Most of the remainder of the lung is collapsed, a picture of atelectasis due to obstruction or to resorption. These changes may be associated with pneumonia in some instances. The occasional petechiae appearing on serous surfaces and in the brain are evidence of asphyxial death.

In contrast to the condition described, the lungs of other newborn infants who have died exhibit a different type of hyaline membrane. This type contains little or no lipid and is most frequently associated with interstitial pneumonia. In the lungs of these infants there are variable amounts of vernix caseosa and plugs of vernix do not appear to occlude the respiratory passages.

## MATERIAL STUDIED

A study was made in two series of 119 consecutive necropsies on infants dying during the period 1938 to 1947. Liveborn infants of both series were all in the neonatal period, i. e., less than thirty days after birth. Permissions to make necropsies were obtained by members of the obstetric and pediatric staffs of the University Hospital.

The original series consisted of necropsies performed on 35 liveborn and 14 stillborn infants during 1946 and 1947. They were performed by the permanent and resident staff of the department of pathology of the University of Georgia.

Hospital charts were carefully examined for contributory causes of death or causes not evident at postmortem examination. The routine hematoxylin-eosin method of staining was followed, with use of Harris' hematoxylin solution. When we suspected that a vernix membrane was present, frozen sections were stained with sudan IV by Herxheimer's method. The first few lungs in which

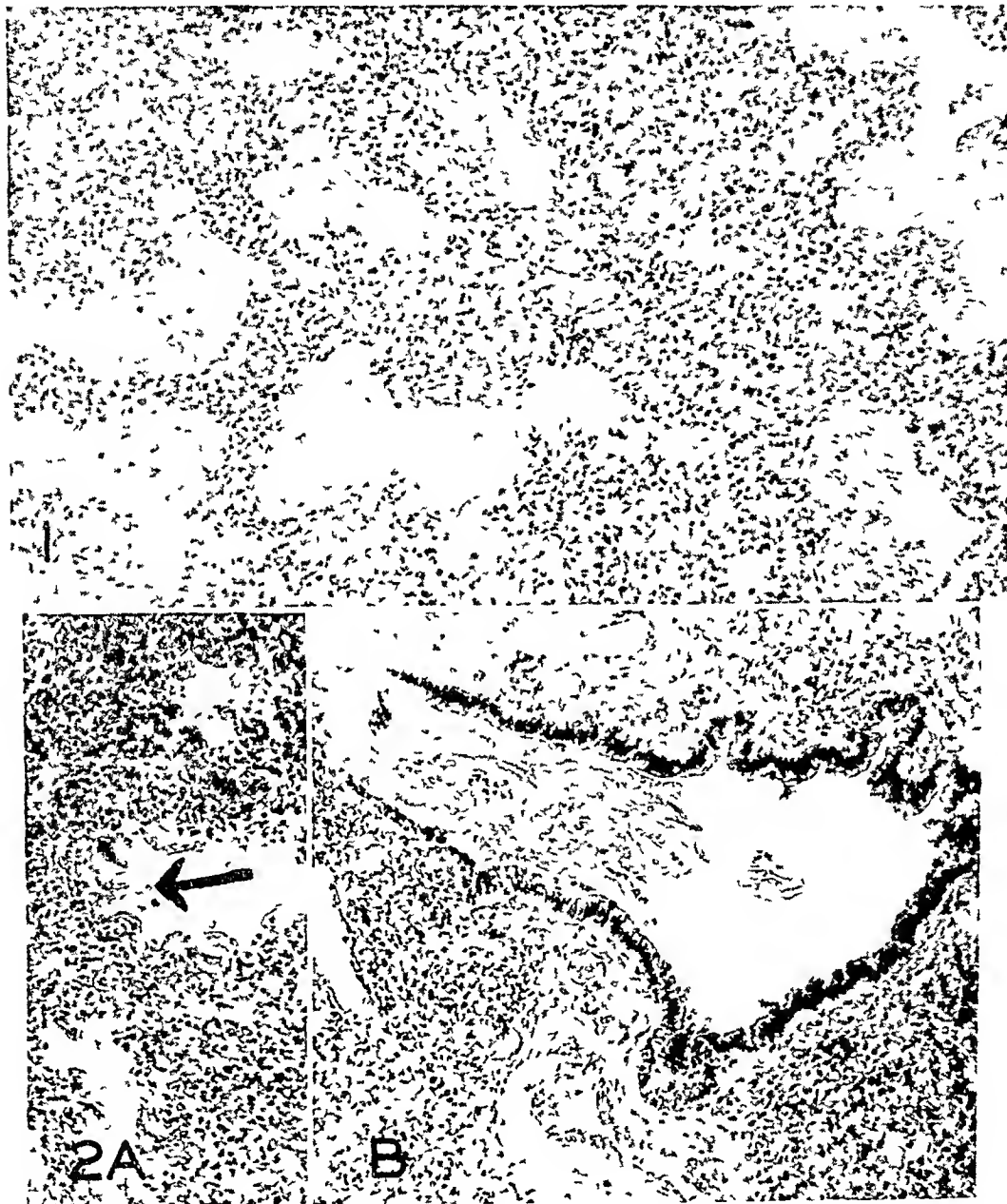


Fig 1—Membrane is seen lining alveolar ducts and sacs. Note extreme resorption atelectasis. Hematoxylin and eosin, low power.

Fig 2—*A*, lipid-rich vernix is seen lining a duct at the alveolar junction. Sudan IV. *B*, lipid-rich vernix is seen plugging a terminal bronchiole. Sudan IV, low power.

a vernix membrane was observed were also stained for mucin. In most instances sections were secured from each lobe of the lungs.

An intrapulmonary vernix membrane was demonstrated in 6 (12 per cent) of the 49 infants. Since the membrane does not occur in stillborn infants, this represents 17 per cent of all the liveborn infants on whom necropsies were made. In 2 instances only the vernix membrane was observed in the lungs. In addition to the membrane 2 infants had congenital pneumonia, and 2 died with an early postnatal pneumonia probably incident to resorption atelectasis of the lungs. There was no other evident cause of death in these infants.

CASE 1—The infant was born at full term. Delivery was normal and spontaneous, with the mother under analgesia induced with intravenously injected scopolamine hydrobromide U S P and pentobarbital sodium U S P. The membranes were intact until shortly before birth. Cyanosis was present at birth. The resuscitation procedure consisted of tracheal aspiration and administration of oxygen with the Torpin insufflator. Synthetic vitamin K (synkamin®) was administered. Little improvement was noted following this therapy. No fever was observed. The infant lived two days.

Microscopic examination of the lungs revealed an extensive vernix membrane and congenital pneumonia.

CASE 2—The infant was born at full term. Delivery was normal and spontaneous with the mother under scopolamine and pentobarbital analgesia. The membranes ruptured at the onset of labor. The infant breathed normally at birth. A few hours later it was cyanotic and was exhibiting a grunting type of respiration. Intratracheal aspiration was performed and oxygen given by means of the Torpin insufflator. The infant improved somewhat, then became more cyanotic. No fever was noted. The infant lived one day.

Microscopic examination showed the lungs extensively involved with a vernix membrane.

CASE 3—The infant was born at full term. Labor was long, and the infant was delivered by low forceps. Respiratory difficulty was noted shortly after birth and breathing became increasingly more labored. The baby became cyanotic and was placed in an oxygen tent. No fever was noted. The infant lived four days.

Microscopic examination showed the lungs moderately involved with a vernix membrane and early pneumonitis.

CASE 4—The infant was born at full term. The mother was given "twilight sleep" and posterior pituitary injection U S P. The membranes were intact until the onset of labor. Breathing was spontaneous, and the infant had good color at birth. Cyanosis developed a short time after birth. Oxygen was given and the color improved. The oxygen gave out, and the baby became cyanotic again. It improved for only a short time when more oxygen was given. No fever was noted. The infant lived twenty-six hours.

Microscopically, the lungs showed an extensive vernix membrane and congenital pneumonia.

CASE 5—The infant was born at full term. The history was incomplete. Respirations were poor at birth. Cyanosis was either present at birth or developed later. The temperature was 96.0 F. Oxygen was given. The infant lived eighteen hours.

Microscopically, the lungs were extensively involved with a vernix membrane.

CASE 6—The infant was born at full term. Labor was of five hours' duration. Scopolamine hydrobromide and pentobarbital sodium were given. Delivery followed tetanic spasm of the uterus and was precipitate. The infant was pale, emaciated.



and pulseless at birth. It later became cyanotic and had grunting respiration, which was deep and pauseless. Intratracheal insufflation of oxygen was given with a Torpin insufflator. The temperature became elevated. The infant lived four days.

Microscopic examination showed the lungs moderately involved with a vernix membrane and early pneumonitis.

The second series studied consisted of 70 consecutive necropsies, the material of which was obtained from the files of this department. It included 45 stillborn and 25 liveborn infants for the period 1938 to 1945 inclusive. One liveborn infant with multiple congenital abnormalities had pulmonary findings consistent with vernix membrane involvement and congenital pneumonia. No sections stained for fat were available. This was 4 per cent of all liveborn infants or 1.4 per cent of all infants, liveborn and stillborn, on whom necropsies were made.

The total number with vernix membrane in the two series therefore was 7 (11.6 per cent) of liveborn infants or 5.8 per cent of the total number of newborn infants on whom necropsies were made. Although the total number of cases of vernix membrane involvement in this series is small for comparison, the incidence is much higher than that reported by Macgregor<sup>7</sup> and Labate<sup>8</sup>.

#### COMMENT

A discussion of asphyxia neonatorum necessitates a short resume of the perplexing problem of fetal respiration and is essential in attempting to show its etiologic relationship to the vernix membrane. Much work has been done on this subject in both human beings and lower animals with varying results. Windle<sup>11</sup> stated that under normal conditions the oxygenation of the fetus is adequate until shortly before term, when there is a progressive decline in placental efficiency. Only at this time do minor rhythms of respiratory movements occur. Normally the fetal musculature is atonic, and therefore it is incapable of initiating sufficient expansion of the lung for aspiration of amniotic contents unless the respiratory or other centers of the central nervous system are excited by a depression of thresholds or an elevation of carbon dioxide in the blood. Should oxygen saturation decrease markedly, numerous motor neurons are activated, muscle tonus increases and dyspneic gasping movements ensue. Surprisingly little activity has been noted in utero in lower animals by Windle. Farber and Sweet<sup>2</sup> expressed the belief that obstruction of placental circulation with resultant intrauterine anoxia initiates premature respiratory movements in utero with aspiration of amniotic contents. Oxidation

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11 Windle, W. F. *Physiology of the Fetus*, Philadelphia, W. B. Saunders Company, 1940.

is thereby further suppressed by obstruction of alveoli and bronchioles Davis and Potter,<sup>12</sup> using the method of Dieckmann and Davis, withdrew amniotic fluid and injected colloidal thorium dioxide into the human amniotic sac. This was done on one series of patients during the first half of gestation and in another series at or near term. Local anesthesia was used, and pregnancy was terminated by elective cesarian section thirty minutes to fifty-two hours after injection. "All infants were alive and normal at birth." In most instances, after death of the fetus, thorium dioxide was found in the lungs by roentgenologic or microscopic examination. In these patients the authors noted spasmodic and irregular respiratory movements of the fetus in utero even in early fetal life. They believed that thorium was concentrated in the lungs as a result of fluid absorption by prealveolar and alveolar capillaries. Much amniotic fluid may escape in this manner, leaving thorium behind. Some of the fluid aspirated into the respiratory tract may escape back into the amniotic cavity by a tidal flow<sup>13</sup> normally present. Potter and Davis concluded that an aquatic existence of the fetus is normal during intrauterine life and that intrauterine respiratory activity is instituted in early pregnancy. Although spasmodic, irregular and shallow, it differs only slightly in pattern from extrauterine respiration, the major change being substitution of air for fluid.

In the present series varying amounts of amniotic contents were found in the lungs of infants known to have experienced intrauterine anoxia or hypoxia. These necropsies performed on both stillborn and liveborn infants included cases of premature separation of the placenta, prolapse of the cord and erythroblastosis fetalis. A vernix membrane was not demonstrated in any of these infants. Damage to the nervous system from anoxia probably accounts for the death of these infants. Therefore, although intrauterine anoxia may lead to exaggerated respiratory movements, this should not be considered the dominant factor responsible for development of the vernix membrane or the presence of much vernix debris, because many newborn infants are subject to varying degrees of intrauterine anoxia and a membrane can be demonstrated in only a few.

Factors other than simple aspiration of contents must be involved whether aspiration occurs normally or abnormally. Several of these factors must be considered. Since the sebaceous glands of certain persons are more active than others, one may assume that this is also true of the fetuses. Desquamation and dissolution of epidermal cells also are probably variable. It is known from clinical observation that the amount of vernix caseosa and amniotic debris in the fluid varies and

12 Davis, M. E., and Potter, E. L. *J. A. M. A.* **131** 1194, 1946

13 Snyder, F. F., and Rosenfeld, M. *Am. J. Obst. & Gynec.* **36** 363, 1938

that the proportion of amniotic debris under ordinary circumstances would vary inversely with amount of fluid. In addition, perhaps vernix may be concentrated not only in the amniotic sac but also in the respiratory passages, since it has been suggested by Davis and Potter<sup>12</sup> that there may be an exchange of amniotic fluid within the lungs by way of alveolar and prealveolar capillaries. If this is true, then vernix caseosa may be excessively concentrated or left behind because of absorption of fluid. Theoretically, then, in the presence of large amounts of concentrated vernix extremely high in lipids, conditions are ripe for development of the vernix membrane.

An attempt was made to produce a membrane in the alveoli of several lungs removed from stillborn infants at autopsy. A sample of amniotic fluid was centrifuged, 5 to 10 cc of the sediment, warmed to body temperature, was injected into a similarly heated lung and alternating negative and positive pressure at 10 mm of mercury applied in a glass chamber until the lung was expanded. In one lung a pseudomembrane formed in some of the respiratory bronchioles, but this membrane contained only small amounts of fat. None was seen in the alveoli. Although constant body temperature was not maintained during the entire procedure, it seems probable that the fluid was not sufficiently high in lipids to produce the characteristic picture. Furthermore, in these lungs there could be no exchange of fluids through capillary walls. Although a negative result was obtained in these instances, it seems to lend support to the foregoing theory. Further work is necessary on this phase of the problem.

In this series there were 13 infants with so-called congenital pneumonia, 7 being stillborn infants. In 3 (50 per cent) of the 6 liveborn infants, the pneumonia was associated with a vernix membrane. Although it is believed by some that the association of pneumonia with the membrane is purely coincidental, the high occurrence of pneumonia in the infants exhibiting the vernix membrane is rather striking. Perhaps the presence of pneumonia causes abnormal stickiness of the cells lining the respiratory passages, such as occurs in endothelium during the phenomenon of inflammation, thereby permitting greater adhesive surfaces on which vernix may be deposited.

In the present series 2 of 5 infants with extrauterine pneumonia lived four days. These exhibited moderate involvement with the vernix membrane. It is well known that atelectasis predisposes to pneumonia, therefore, in these cases the membrane seems to be a definite predisposing factor.

Although Farber and Sweet<sup>2</sup> expressed the belief that there is no reaction in the lungs to amniotic contents and Johnson and Meyer<sup>1</sup> stated that a slight reaction may occur, there remain those patients

who might live with moderate amounts of vernix in the form of a membrane Nelson and Venable,<sup>14</sup> however, reported a case in which a patient with symptoms at birth suggestive of asphyxial membrane involvement died at 14 months with acute bacterial endocarditis. The lungs at necropsy showed many alveoli obliterated by fibrous connective tissue and the walls of functioning ones thickened, "probably a result of amniotic fluid pneumonia." Of course, it is impossible to state that this "pneumonia" was in reality one of the vernix membrane type but it is interesting to speculate on that possibility in this case.

Finally, what is the relationship between modern methods of resuscitation and development of the membrane? We have seen that, following cesarian section, if adequate immediate aspiration is done, the mortality rate may be lowered from 10 to 2 per cent. This lower mortality rate was apparently, for the most part, in a "pseudo-membrane" group of cases. This suggests that immediate intratracheal aspiration may, in some cases, prevent aspiration of excessive amounts of amniotic debris and more extensive membrane involvement.

It was thought that active resuscitation under normal pressures with the Torpin insufflator<sup>15</sup> might have increased the incidence of the membrane in the 1946-1947 series. However, the insufflator has been in use during the period of this entire series. Furthermore, insufflation is done on most stillborn infants and no membrane has yet been encountered. Therefore there is a discrepancy in the percentage with membrane involvement in the two groups which is unaccounted for because of this factor<sup>16</sup>. During the past two years infants have been placed in the bassinet with head elevated instead of depressed. It is thought this position may have led to poor drainage of the respiratory tract in cases examined during that period.

#### SUMMARY AND CONCLUSIONS

A vernix membrane forms in the lungs of a significant number of liveborn infants, which leads to asphyxia within a few hours to four days after birth. Although a high percentage also have pneumonia, there is usually no other obvious cause of death.

This membrane prevents exchange of gases in opened alveoli and is associated with vernix in terminal bronchioles and alveolar ducts which produces atelectasis due to obstruction and resorption.

14 Nelson, R. L., and Venable, D. R. *J. Lab. & Clin. Med.* 26: 772, 1941.

15 Volpito, P. P., and Torpin, R. *South. M. J.* 35: 559, 1942.

16 Since this present series was completed a newborn infant has been encountered who never breathed spontaneously. Artificial respiration was given for four hours by means of the Torpin insufflator, and the heart continued to beat for that period of time. An intrapulmonary vernix membrane was found in this infant and was associated with pneumonia.

In these infants the vernix is extremely high in lipids and is present in large amounts in the respiratory tract and alveoli before birth.

In these infants the vernix may have been concentrated in the lung either because of absorption of fluid by way of exchange through alveolar and prealveolar capillaries or because only small amounts of amniotic fluid were present.

The presence of the membrane may represent only a variant of the aspiration of amniotic debris and vernix caseosa so frequently found to be a cause of asphyxia neonatorum.

Following the first extrauterine breath, this material in part is forced against the walls of alveoli and alveolar ducts, while that present in the upper respiratory tract lodges in the respiratory bronchioles. As extrauterine respirations become more forceful, greater expansion takes place, and more vernix is aspirated into the smaller units from above.

Its high incidence in association with congenital pneumonia is probably due to the increased stickiness of the respiratory passages which has developed during the inflammatory process.

Intrauterine anoxia may produce exaggerated intrauterine respiratory movements with aspiration of greater amounts of amniotic fluid, but death in that case is primarily due to damage within the central nervous system.

It is postulated that the necessary factor concerned with development of the membrane is concentration of vernix which has an extremely high lipid content. Pneumonia prepares a fertile field for its development. Exaggeration of intrauterine respiratory movements is a factor only in those cases presenting the aforementioned prerequisites.

Aspiration may be of no avail in many cases when much of the vernix is beyond the tracheal bifurcation, but in others it may forestall more extensive involvement of the lung, and death of the infant may be prevented.

# IN VITRO STUDIES OF LYMPH NODES INVOLVED IN HODGKIN'S DISEASE

## I Liquefaction of Culture Medium

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**T**ISSUE cultures of lymph nodes involved in Hodgkin's disease develop according to a pattern peculiar to, but not specific for, this disease. The pattern consists of the following three easily demonstrated and frequently recurring phenomena: (1) evolution of giant cells, (2) intracytoplasmic inclusions, (3) liquefaction of areas of the culture medium. We have studied all three of these phenomena and wish at this time to present our analysis of the liquefaction of the medium.

### HISTORICAL REVIEW

In 1921 Lewis and Webster<sup>1</sup> reported their study of cultures of human lymph nodes grown in vitro. They noted that liquefaction occurred on the first and second days after implantation but made no further comment on it. A year later Maximow<sup>2</sup> stated that in cultures of rabbit lymph nodes the liquefaction of fibrin commonly observed in cultures of epithelium did not take place. The first comprehensive report of in vitro studies of Hodgkin's disease was made by Mankin,<sup>3</sup> in 1936. He observed that liquefaction did not occur in cultures of tuberculous nodes and did not occur, as a rule, in cultures of leukemic nodes but that it was prominent in cultures of lymph nodes involved in Hodgkin's disease and that it usually began on the sixth or the seventh day. In 1937 Meier<sup>4</sup> presented his findings in cultures of nodes affected by Hodgkin's disease but made no mention of liquefaction. Like Mankin, Grand<sup>5</sup> observed liquefaction during the course

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From the Laboratory for Hodgkin's Disease Research, St Vincent's Hospital

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1 Lewis, W H, and Webster, L T. *J Exper Med* **33** 261, 1921

2 Maximow, A. *Arch f micr Anat* **96** 494, 1922

3 Mankin, Z W. *Beitr z path Anat u z allg Path* **96** 248, 1936

4 Meier, R, Posern, E, and Weizmann, G. *Virchows Arch f path Anat* **299** 329, 1937

5 Grand. Personal communication to the authors

of his studies of tissue cultures of lymph nodes from patients with Hodgkin's disease. The primary concern of his preliminary studies was the intracytoplasmic inclusions and the possibility that these were related to virus activity, but in later studies he concentrated on liquefaction and the possibility that it was related to an etiologic agent. He demonstrated that liquefaction of medium could be induced by adding drops of extracts of the diseased lymph nodes to cultures of chick chorioallantois.<sup>5</sup> During the period 1945 to 1947 Hoster<sup>6</sup> noted liquefaction when normal guinea pig spleen was grown in medium to which cell-free extracts and sediments of diseased tissue had been added, liquefaction also occurred in the controls, but much less often.

In our study we were chiefly interested in (1) the specificity of the liquefaction, (2) the degree of liquefaction produced by fragments of nodes in different histologic stages of Hodgkin's disease, (3) the degree to which liquefaction is related to the clinical picture, (4) the dynamics and the mechanism involved in producing the phenomenon.

#### MATERIAL AND METHOD OF STUDY

Fifty-six lymph nodes were studied, 29 from 26 patients with Hodgkin's disease (table 1) and 27 from 27 patients who did not have Hodgkin's disease (table 2). Two of the patients with Hodgkin's disease had two biopsies each and 1 patient had three biopsies, all being performed at six to twelve month intervals. After removal the lymph nodes were placed in Ringer's solution and cultivated within one-half hour. Each node was first rinsed with a solution of penicillin (500 units per cubic centimeter in Tyrode's solution) and then sectioned. One portion was kept for histologic study, the other, for tissue culture. After thorough washing with Tyrode's solution, small fragments were cut from representative portions of the node and cultivated according to Maximow's double cover slip method. The medium consisted of 1 volume of adult chicken plasma, 3 volumes of human placental cord serum and 1 volume of chick embryo extract. Two or 3 drops of this mixture were sufficient to make a clot on the small cover slip. Usually two fragments were embedded in each clot and incubated at 37.5 C. Every two or three days the cultures were washed in warm Tyrode's solution for twenty minutes, and one drop of the original medium was placed on the surface of the clot. From 20 to 60 implanted pieces of tissue from one node constituted a series. At twenty-four hour intervals, two or more slides were fixed in Carnoy's solution, Zenker's formol or Ringer's formol.<sup>6a</sup> As a rule, the experiment was terminated at the end of seven days, since we had found by experience that in this interval of time most of the explants and their outgrowths had been replaced by fibroblasts.

#### RESULTS

Usually by the second or the third day a ringlike area, or zone of liquefaction was found at one side of the explant. Mankin called this a "liquefaction vacuole," and we, also, shall so designate it (fig 1). When first observable the phenomenon suggests that a thin margin is separating from the periphery of the explant.

6 Hoster. Personal communication to the authors.

6a R. C. Parker. *Methods of Tissue Culture*, New York, Rockefeller Institute, p. 171.

(fig 2) This margin becomes displaced for a variable distance beyond the parent explant, but, as a rule, the explant and the displaced margin remain connected at two opposite points (fig 1, *E-E*), thus there is formed a boundary about a semilunar or roughly spherical area in which the medium, previously solid, has become a fluid in which the cell population can be seen floating about. This population must be differentiated from the cells floating in fluid expressed by the clot during gelation. If the culture is washed at this time, these cells float away, and a clear area is left behind. The displaced marginal fragment is composed of cell elements the same as those present at the intact surface of the explant, namely, lymphocytes, reticulum cells and also fibroblasts, provided displacement has taken

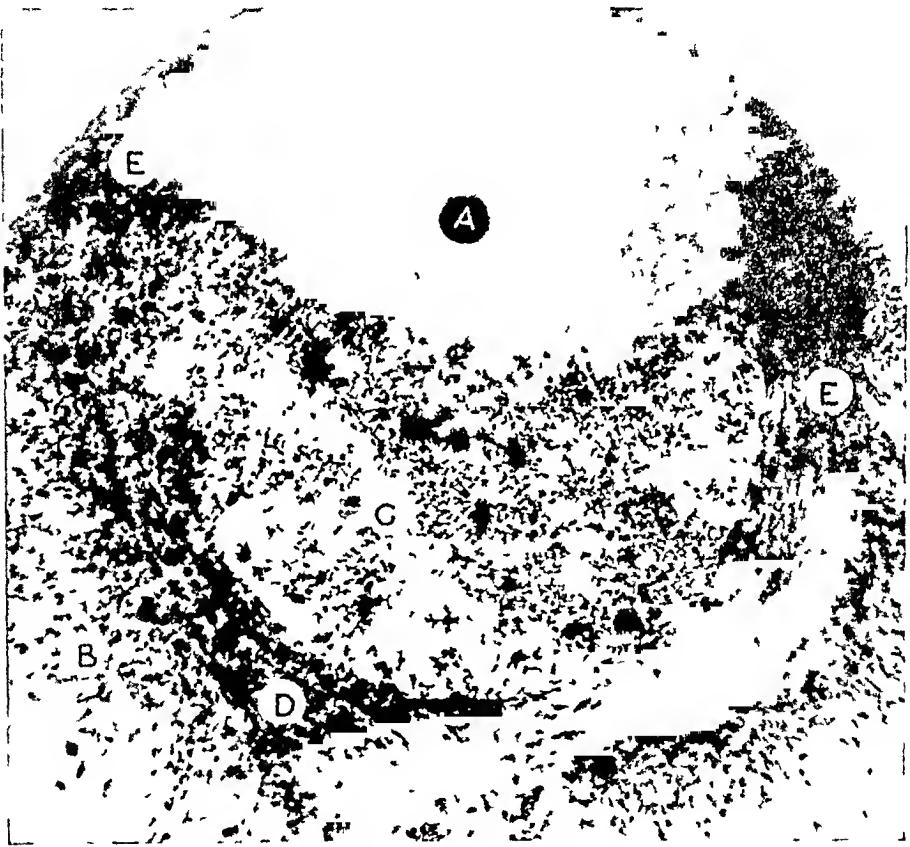


Fig 1—Photomicrograph of a fragment of a lymph node involved in Hodgkin's disease in which a liquefaction vacuole is fully formed. *A*, is the tissue fragment, *B*, the outgrowth or zone of migration, *C*, the area of liquefaction, *D*, the displaced margin of the fragment, *E-E*, the points at which the displaced margin of the fragment maintains continuity with the parent tissue.

place subsequently to the time at which the fibroblast-like cells develop in the explant (usually on the third or the fourth day). The displaced margin appears to rest on solid, unchanged medium. Within the liquefaction vacuole there is a mixed population made up usually of reticulum cells, lymphocytes and multinuclear giant cells, though these constituents may vary in different series, on different slides and in two explants on the same slide. Also within the liquefaction vacuole there is considerable activity, such as immigration of new cells coming from the explant, cell multiplication, cell degeneration and phagocytosis. In those cases in which the



vacuole forms before fibroblastic proliferation has begun, the fibroblasts, after their advent, begin to migrate from the explant and, creeping along the border of solid medium, they completely encircle this at its periphery. They continue to proliferate, arrange themselves so as to form an ever-thickening capsule and then invade the area of liquefaction (fig 3). By the seventh day or later this area has become completely filled and is indistinguishable from areas not previously liquefied. These so-called liquefaction vacuoles vary in size, shape and number. As a rule, each explant develops but one, occasionally as many as three are found.

Having noted the dynamic histologic pattern of the liquefaction, we next concentrated attention on the specificity and the constancy of the liquefaction

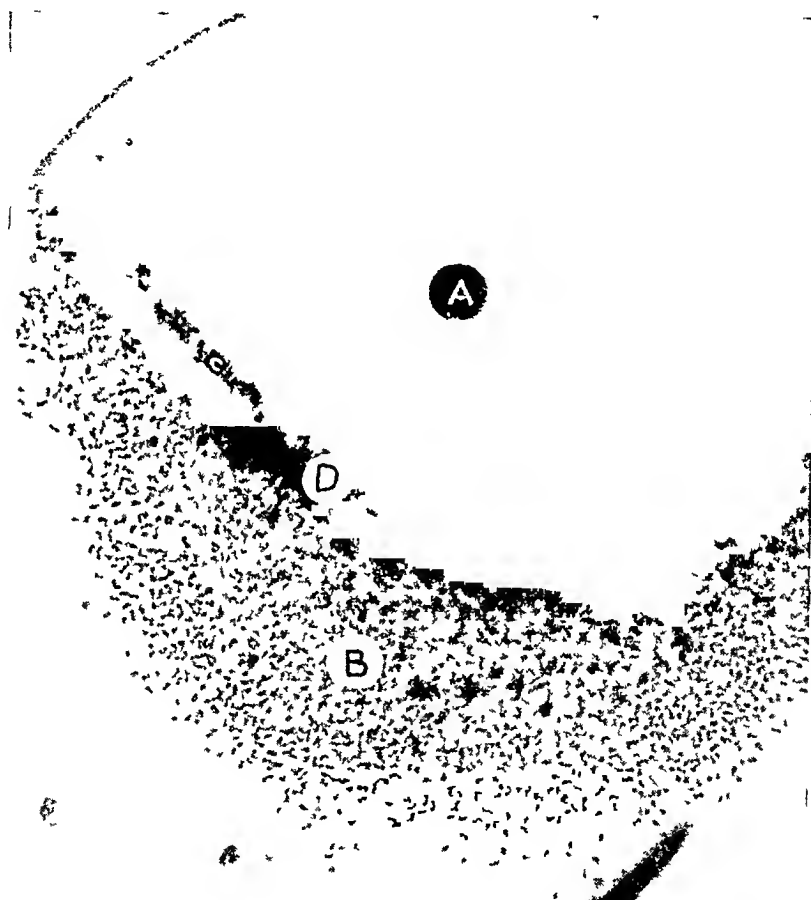


Fig 2—Photomicrograph showing an early stage of the formation of the liquefaction vacuole, which at this time is a narrow slit (C). The other letters in this and other figures to follow are explained in the legend for figure 1.

produced by the diseased nodes when these were grown in vitro. Soon it became apparent that neither was the liquefaction specific for Hodgkin's disease (fig 4), nor did it occur constantly (table 1). Nevertheless, it was produced much more often by the nodes involved in Hodgkin's disease than by nodes not involved in this disease, grown under identical conditions (tables 1 and 2). Tables 1 and 2 show that 62 per cent of Hodgkin's disease cultures underwent liquefaction of medium, compared with 33 per cent of the cultures of nodes involved in diseases other than Hodgkin's disease. Cultures of tuberculous nodes also had a high incidence of liquefaction. In 5 cases of malignant lymphoma (2 of lymphocytic

TABLE 1—Data on Liquefaction of Medium of Cultures of Lymph Nodes Affected by Hodgkin's Disease

| Specimen                                | 11   | 12 | 13 | 14  | 15 | 16 | 17 | 18 | 19 | 20 | 21* | 22* | 23† | 24† | 25† | 26† | 27† | 28 | 29 |
|---|------|----|----|-----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|----|----|
| Explants per series                     | 115‡ | 56 | 22 | 7   | 22 | 47 | 44 | 35 | 38 | 21 | 18  | 13  | 37  | 49  | 67  | 37  | 29  | 1  | 2  |
| Explants showing liquefaction           | 0    | 35 | 6  | 7   | 5  | 10 | 26 | 17 | 16 | 4  | 9   | 1   | 4   | 6   | 0   | 8   | 7   | 1  | 2  |
| Percentage showing liquefaction         | 0    | 62 | 27 | 100 | 22 | 21 | 53 | 48 | 42 | 19 | 50  | 7   | 10  | 12  | 0   | 21  | 25  |    |    |
| Day liquefaction appeared               |      | 1  | 2  | 5   | 3  | 3  | 2  | 2  | 4  | 3  | 4   | 7   | 2   | 2   | 0   | 2   | 3   |    |    |
| Histologic diagnosis of disease of node | 7 G  | G  | G  | G   | GF | G  | G  | G  | GF | G  | PG  | PG  | G   | G   | G   | G   | G   | PG | G  |
|   | 1 PG |    |    |     |    |    |    |    |    |    |     |     |     |     |     |     |     |    |    |
|   | 3 S  |    |    |     |    |    |    |    |    |    |     |     |     |     |     |     |     |    |    |

\* Specimens 21 and 22 were taken from the same patient at two biopsies made at different times

† Specimens 23, 24 and 25 were taken from the same patient at three biopsies made at different times

‡ Specimens 26 and 27 were taken from the same patient at two biopsies made at different times

G stands for the granulomatous form of Hodgkin's disease, PG, for the pregranulomatous form of Hodgkin's disease S, for the sarcomatous form of Hodgkin's disease, GF, for the granulomatous form of Hodgkin's disease, fibrotic stage

TABLE 2—Data on Liquefaction of Medium of Cultures of Lymph Nodes Affected by Diseases Other Than Hodgkin's Disease When Representative Fragments of the Nodes Were Grown in Vitro

| Specimen                        | Nonspecific Hyperplasia |    |     | Tuberculosis |    |    |    |    | Anthracoosis |    |    | Lymphoma |     |     |     |     | Metastatic Carcinoma |     |    |    |
|---------------------------------|-------------------------|----|-----|--------------|----|----|----|----|--------------|----|----|----------|-----|-----|-----|-----|----------------------|-----|----|----|
|                                 | 1                       | 2  | 3   | 11           | 12 | 13 | 14 | 15 | 16           | 17 | 18 | 19       | 20* | 21† | 22† | 23† | 24†                  | 25† | 26 | 27 |
| 1 explants per series           | 38                      | 36 | 270 |              | 50 | 28 | 36 | 30 | 40           | 20 | 22 | 22       | 18  | 48  | 7   | 40  | 64                   | 40  | 11 | 60 |
| 1 explants showing liquefaction | 3                       | 1  | 0   |              | 17 | 10 | 4  | 0  | 0            | 4  | 0  | 0        | 0   | 0   | 4   | 9   | 0                    | 0   | 1  | 0  |
| Percentage showing liquefaction | 7                       | 2  | 0   |              | 34 | 33 | 11 | 0  | 0            | 20 | 0  | 0        | 0   | 0   | 0   | 57  | 22                   | 0   | 9  | 0  |
| Day of appearance               | 5                       |    | 0   |              | 3  | 5  | 4  | 0  | 0            | 3  | 0  | 0        | 0   | 0   | 3   | 2   | 0                    | 0   | 0  | 0  |

\* Specimen 20 was involved in lymphatic leukemia

† Specimens 21, 22 and 24 showed reticulum cell sarcoma

‡ Specimens 23 and 25 showed lymphosarcoma

sarcoma, 3 of reticulum cell sarcoma) it occurred twice, or in 40 per cent. If the Hodgkin's disease group is compared with the non-Hodgkin's disease group with respect to the relative incidence of liquefaction, the following figures are obtained: 164 of the 445 Hodgkin's disease cultures, or 37 per cent, and 53 of the 226 non-



Fig 3—An area of liquefaction being overgrown with fibroblast like cells. By the fourteenth day such areas may become completely filled.

Hodgkin's disease cultures, or 19 per cent, underwent liquefaction. In this calculation we did not include culture series, normal or diseased, showing no liquefaction whatever.

An attempt was next made to determine whether nodes with one histologic type of Hodgkin's disease produced liquefaction more often than nodes with a different histologic type. The only impressive finding was that cultures of the sarcomatous nodes failed to liquefy. All of the cultures of the more fibrous Hodgkin's disease nodes and about two thirds of those of the lymphocytic and of the granulomatous type did liquefy. No correlation could be established between this peculiar behavior of the node grown *in vitro* and the duration of disease, the tempo of disease or the clinical symptoms observed. In 3 instances multiple biopsies were performed. The third node removed from one patient failed to produce any cultures which liquefied, a second node from another patient gave a series which showed marked reduction of the number of cultures which liquefied—7 per cent, compared with 50 per cent of the cultures from the first node—and two nodes from a third patient produced an equal number of cultures in which liquefaction occurred.

#### COMMENT

When lymph nodes involved in Hodgkin's disease are grown *in vitro* under the conditions outlined, liquefaction of medium frequently occurs. This phenomenon, however, is neither peculiar to, nor specific for, Hodgkin's disease, for the same behavior is exhibited by cultures of lymph nodes affected by other diseases, namely, tuberculosis, lymphosarcoma, anthracosis, metastatic carcinoma and simple lymphoid hyperplasia. Despite this lack of specificity, however, the lymph nodes involved in Hodgkin's disease produce liquefaction with sufficient frequency to warrant ascribing significance to it, even though this significance is at present not understood. The fact that liquefaction occurs in tissue cultures of nodes affected by nonrelated diseases points to the existence of some common fundamental biologic mechanism latent in all nodes, that which renders the understanding of this mechanism perplexing is the extreme irregularity with which it is set into motion. Why, for instance, did only 1 of 37 fragments of a hyperplastic node exhibit liquefaction? Why did 35 of 56 fragments liquefy in one instance of Hodgkin's disease and only 5 of 22 in another? The most obvious way in which to begin an attempt to explain these facts seemed to be to relate this phenomenon, if possible, to the varying histologic aspects of the parent lymph nodes. When this had been done, no criterion emerged by which one could predict whether liquefaction would or would not take place in a given series of cultures. Comparison of the tissue culture behaviors of multiple nodes taken at different intervals from each of 3 different patients obscured the matter still further, since nodes from the same patient exhibited different behaviors even though the histologic aspects were similar.

Since the medium used in our tissue cultures was a fibrin coagulum the obvious explanation of its liquefaction would be action by a fibrin-

olytic enzyme. A similar explanation was offered by Santesson,<sup>7</sup> who studied liquefaction occurring in cultures of epithelial tissue. We have not yet made a systematic study of enzymosis, but attempts to demonstrate proteolytic enzymes in lymphocytes have already been made by several workers. In 1908 Longcope and Donhauser<sup>8</sup> demonstrated that proteolytic enzymes were active, in acid medium, in the cells of a large cell lymphatic leukemia and absent in small lymphocytes, Morris and Boggs<sup>9</sup> (1909) extracted a protease from small lymphocytes obtained from a patient with chronic lymphatic leukemia and found the enzyme to be active in a neutral medium, Rona and Kleinmut<sup>10</sup> studied proteolytic properties of a lymph node and of spleen extracts and found kathepsin, active at  $p_H$  4 to 5, and traces of trypsin, active at  $p_H$  7 to 8, in rabbit lymphocytes. Barnes<sup>11</sup> found small quantities of nuclease, amylase, lipase, lysozyme and adenosinase, and from cat lymphocytes he obtained kathepsin, nuclease, lipase and lysozyme. Suggestive preliminary studies, with tissue cultures as a test medium, have already been made by Grand.<sup>6</sup> He added extracts of lymph nodes affected by Hodgkin's disease in vitro to cultures of chick chorioallantois and obtained liquefaction of the medium. The same extracts added to medium alone, devoid of any implant, were said not to induce liquefaction. This would suggest that if enzymes were contained in the extracts they were inactive on the fibrin clot in the absence of living tissue, and this supposition seems supported by our studies, for in no instance did liquefaction occur when the fragment failed to grow.

Thus the *vis a tergo* for liquefaction of medium resides in the living cells of the fragment and is dependent for its operation on the active growth of these cells. In addition to this, there seems to be a second factor contributing to liquefaction—a factor which probably is related to conditions prevailing at certain regions of the periphery of the explant, since it always occurs there. Whether the activity begins immediately within or immediately outside the margin of the explant is difficult to determine, but at any rate some stretches of the periphery are favorable to reaction to an unknown stimulus and some are not. One of the favorable conditions may well be an optimum hydrogen ion concentration. This and other possible factors merit further study.

Also to be determined is which cell or cells growing in the culture are responsible for the elaboration of the liquefying factor—the lympho-

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7 Santesson, L. *Acta path et microbiol Scandinav*, 1935, supp 24, p 85

8 Longcope, W T, and Donhauser, J L. *J Exper Med* **10** 618, 1908

9 Morris, R S, and Boggs, T R. *Arch Int Med* **8** 806, 1911

10 Rona, P, and Kleinmut, H. *Biochem Ztschr* **241** 283, 1931

11 Barnes, J M. *Brit J Exper Path* **21** 264, 1940

cyte, the reticulum cell, the multinuclear giant cell or the fibroblast. In various cultures which came under our observation we frequently



Fig 4—A liquefaction vacuole forming in a fragment of a tuberculous lymph node

noted lymphocytes growing out from one side of the explant in dense, compact masses or diffusely from the entire periphery. Although

these underwent disintegration, no liquefaction occurred, or if it did occur, it appeared in areas other than those in which the disintegrating cells in question were present. The same observation was made in the case of the reticulum cells. This would indicate that under the conditions present neither of these types of cells elaborated a ferment capable of effecting lysis. One may not conclude from this that neither the reticulum cell nor the lymphocyte is capable of producing the enzyme, for in one series of cultures made from a lymphocytic lymphosarcoma, a sarcoma composed of small, mature lymphocytes, 9 of 22 cultures exhibited liquefaction, and in another series, made in a case of reticulum cell sarcoma, 4 of 7 implants liquefied. The fibroblast can be dismissed as a possible agent, since liquefaction never occurred late in the life of the culture, when fibroblastic proliferation was most vigorous. For a time we were inclined to ascribe the phenomenon to the multinuclear giant cells because of the fact that these were so conspicuously present in the liquefaction vacuoles. However, we found many zones of liquefaction free of giant cells and observed giant cells present in nonliquefied areas. The fact that formation of multinuclear giant cells was so frequently associated with liquefaction of medium suggests that some sort of interrelationship exists. Perhaps the factor which induces reticulum cells to transform into giant cells also induces them to elaborate fibrinolytic substances. Finally it should be added that even though liquefaction is a phenomenon nonspecific for Hodgkin's disease (as we believe our experiments have demonstrated) it must not therefore be assumed that liquefaction is always evidence that some one particular etiologic agent is at work. In pathologic tissues one frequently observes that varied and nonrelated stimuli induce similar reactions, the same may be true of tissue reactions observed *in vitro*.

#### SUMMARY

Liquefaction of medium occurs with sufficient frequency in cultures of lymph nodes involved in Hodgkin's disease, when representative fragments are grown *in vitro*, to warrant ascribing to it some significance.

The phenomenon is not specific, since it also occurs in cultures of lymph nodes that are affected by diseases other than Hodgkin's disease. This does not necessarily indicate the existence of a particular etiologic agent responsible for liquefaction.

It seems probable that liquefaction is due to an enzyme, but no biochemical studies have yet been undertaken to establish this as a fact.

It cannot be stated as a fact that the hypothetic ferment is induced by the lymphocytes or by the reticuloendothelial cells, singly or in combination, but it seems to us that the reticulum cell may possibly be the agent responsible

We are of the opinion that certain favorable conditions must be created in order for liquefaction to take place and that these conditions occur at the periphery of living and actively growing explants

There is no relationship between the occurrence and the degree of liquefaction of medium, on one hand, and the histologic aspects of the lymph node and the clinical aspects of Hodgkin's disease, on the other



# IN VITRO STUDIES OF LYMPH NODES INVOLVED IN HODGKIN'S DISEASE

## II Tissue Culture Studies, Formation, Behavior and Significance of the Multinucleated Giant Cell

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**I**N A previous communication<sup>1</sup> it was stated that when grown in vitro lymph nodes affected by Hodgkin's disease displayed certain phenomena which, though nonspecific, were nonetheless striking and of possible significance. In the communication referred to, one of these phenomena, namely, liquefaction of medium, was discussed in detail. We now wish to describe and discuss the possible significance of a second phenomenon—the occurrence of multinucleated giant cells.

### REVIEW OF THE LITERATURE

In tissue cultures of nodes involved in Hodgkin's disease, tuberculous nodes and nodes showing chronic nonspecific adenitis, Lewis and Webster<sup>2</sup> observed giant cells similar to those we shall describe. Several years later Lewis<sup>3</sup> reviewed the subject of the giant cells that form in tissue cultures, the frequency of their occurrence and the lack of relationship to any specific disease, and demonstrated that their appearance was limited neither to lymph nodes nor to any one species of animal. He observed multinucleated giant cells alike in cultures of spleen and of marrow from human beings, mice, rats, chickens, rabbits, guinea pigs, dogs, fish, amphibia and reptiles, and in cultures of cells of buffy coats prepared from blood specimens of this same group of animals. Regardless of whether cells for culture were obtained from buffy coat or from tissue fragments of spleen or lymph node, and regardless of the species of animal from which these were procured, the giant cells emerging from them in vitro appeared to be alike.

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From the Hodgkin's Disease Research Laboratory, St Vincent's Hospital.  
This investigation was supported by a grant from the Research Grants Division of the National Institute of Health.

1 Rottino, A, and Hollender, A. Arch Path, this issue, p 317

2 Lewis, W H, and Webster, L T. J Exper Med **33** 261, 1921

3 Lewis, W H. Am Rev Tuberc **15** 616, 1927

Mankin,<sup>4</sup> in his tissue culture of Hodgkin's disease nodes, also noted multinucleated giant cells, which made their appearance on or about the seventh day, gradually increased in numbers and then degenerated in eight to ten days. They sometimes attained large size and developed as many as 80 to 100 nuclei. It was Mankin's opinion that the multinucleated giant cells under discussion developed from reticular cells and bore no direct histogenic relationship to Sternberg-Reed cells. He noted too that the multinucleated giant cells occurred more often in cultures of Hodgkin's disease nodes than in cultures of nodes not affected by Hodgkin's disease.

Meier,<sup>5</sup> too, found giant cells conspicuous in tissue cultures prepared from Hodgkin's disease nodes. He failed, however, to differentiate between various types of giant cells and hence, when he refers to "giant cells" as possible Sternberg-Reed cells, confusion arises as to the precise cells to which he is referring.

Grand<sup>6</sup> denominated the type of multinucleated giant cell under discussion in the present paper as the Sternberg-Reed cell. Stout<sup>7</sup> agreed with this opinion and added that the cells began as typical Sternberg-Reed cells and that after migrating from the explant, they change their appearance and resemble thereafter foreign body giant cells.

Hoster<sup>8</sup> observed multinuclear giant cells in tissue cultures of normal uninoculated cells. He concluded that they were not specific but represented in some instances a reaction to a contaminant—chicken lymphoma virus—introduced into the culture medium via chicken embryo extract and chicken plasma obtained from flocks known to have a high incidence of lymphomatosis. In other instances the giant cells, according to Hoster, represent a reaction to cell-free Hodgkin's disease material added to normal cells in tissue culture.

Our interpretation has been at such variance with these later opinions that we feel it pertinent to present our views and the findings on which they are based.

#### MATERIAL

The material and the method used in the present study were outlined in our first paper<sup>1</sup>. Briefly, they were as follows. Twenty-eight lymph nodes from 28 persons who did not have Hodgkin's disease and 27 nodes from 23 patients with Hodgkin's disease were used. A series of cultures were made from each node, Maximow's double cover slip method being used. At twenty-four hour intervals

4 Mankin, C. W. *Beitr. z. path. Anat. u. z. allg. Path.* **96** 248 and 308, 1936.

5 Meier, R., Posern, E., and Weizmann, G. *Virchows Arch. f. path. Anat.* **299** 329, 1937.

6 Grand, C. G. (a) *Proc. New York Path. Soc.*, 1945, p. 137, (b) *Proc. Soc. Exper. Biol. & Med.* **56** 229, 1944.

7 Stout, in discussion of Grand<sup>6a</sup>.

8 Hoster, H. Personal communication to the author.

slides with their cultures were fixed in various fixatives and stained with Giemsa stain. That the multinucleated giant cell is of some significance in Hodgkin's disease was deduced from statistical evaluation of comparative observations of the Hodgkin's disease and non-Hodgkin's disease groups.

#### OBSERVATIONS

The giant cell under discussion is a large, multinucleated structure of varying size and shape. Some specimens are of enormous size (fig 1), thin, spread out, with barely perceptible margins, while others are smaller, more oval, with visible borders (fig 2). Conspicuous in the giant cell is the large number of nuclei—sometimes as many as 100 or more—arranged about a central hyperchromatic mass which, when decolorized, is seen to contain particulate matter, the particles being of various sizes. If the hyperchromatic mass is absent, there is a more diffuse distribution of the nuclei. Within the cytoplasm vacuoles containing ingested cells and cell fragments are frequently found.

The multinucleated giant cells are usually seen on the third and fourth days of the culture—earlier in some instances, later in others. They appear in the central fragment, in the zone of migration and in the liquefaction vacuole. New giant cells are subsequently observed until the tenth to fourteenth day, or later, depending in part on when the culture becomes overgrown with fibroblasts. The life span of giant cells varies. It may be as short as three days. While young the cells are barely visible, within twenty-four hours they become more distinct and their cytoplasm becomes granular, from forty-eight hours on they shrink and finally disintegrate.

Typical Sternberg-Reed cells were indubitably identified in cultures of only a few Hodgkin's disease nodes. In these cultures they bore strong resemblance to the Sternberg-Reed cell seen in tissue sections of nodes removed at biopsy and hence they easily could be distinguished from the large multinucleated giant cells under discussion.

Having satisfied ourselves as to the histologic aspects of the giant cell and having identified it as morphologically different from the Sternberg-Reed cell, we next considered its specificity and attempted to find quantitative and qualitative differences in its behavior which would furnish culture criteria for differentiating Hodgkin's disease from other forms of adenitis and lymphoma.

It quickly became apparent that the giant cell in question is not peculiar to any specific disease affecting the node, since it occurred in cultures of hyperplastic nodes and tuberculous nodes as well as in cultures of Hodgkin's disease nodes and lymphoma of other types. Quantitative differences were, however, noted. In the Hodgkin's disease group, giant cells were found in cultures of more nodes and in more cultures from each node, and were present in greater numbers per fragment cultured, than was the case in the non-Hodgkin's disease group. Of 27 Hodgkin's disease nodes, 16 (59 per cent) produced giant cells as compared with 9 of 28 (32 per cent) of non-Hodgkin's disease nodes. Both nodes and fragments which produced multinuclear giant cells in tissue culture will be referred to hereafter as "positive." The 16 positive Hodgkin's disease nodes produced 180 positive fragments (31 per cent of the 590 planted). The 9 positive non-Hodgkin's disease nodes produced only 30 positive fragments (13 per cent of the 228 planted). The total number of giant cells in the positive fragments of Hodgkin's disease nodes was 4,349 against 335 in the non-Hodgkin's disease nodes. This meant that the average Hodgkin's disease positive fragment contained about 23 giant cells, contrasted with 11 giant cells contained by the non-Hodgkin's disease fragments.



Fig 1—Photomicrograph of a multinucleated giant cell observed in tissue culture of a fragment of a lymph node from a patient with Hodgkin's disease. The cell is large, spread out thin; the outline is indistinct; the cytoplasm, pale; the nuclei are numerous and disorderly in arrangement. Present in the cytoplasm are other smaller, rounder and more hyperchromatic nuclear masses representing remains of ingested cells, such as lymphocytes.

Close scrutiny of the giant cells revealed certain detectable cytologic differences between the two groups. For instance, in cultures of Hodgkin's disease nodes more of the giant cells were apt to be larger and to contain greater numbers of nuclei. No differences in phagocytosis could be detected, in both groups many multinucleated giant cells were actively phagocytic.

Attempts to correlate the clinical state of the patient with Hodgkin's disease and the duration and tempo of the disease with giant cell behavior proved unsuccessful.

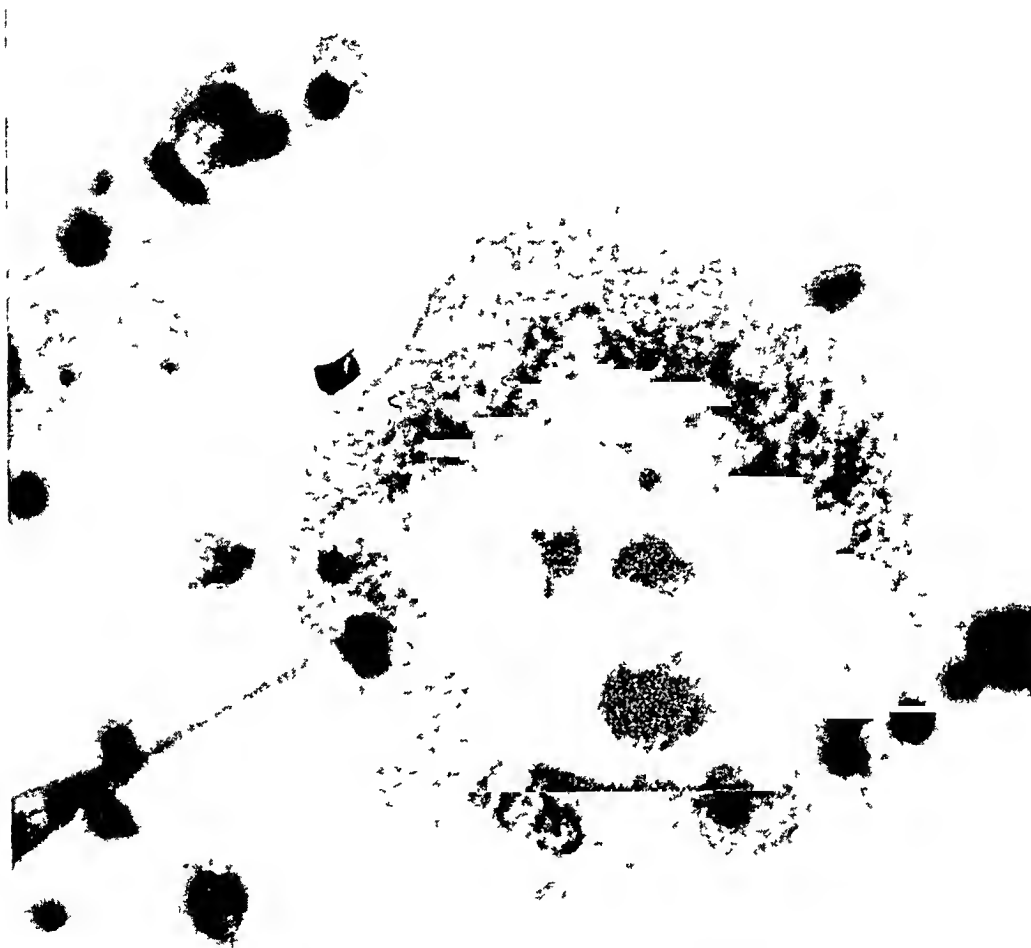


Fig 2—Another form of multinucleated giant cell from a preparation similar to that from which the giant cell of figure 1 was taken. The cell is smaller, more compact, the outline is more distinct, the nuclei are arranged about a central hyperchromatic mass. Both inside this mass and outside it dark, round nuclear remains of ingested cells are to be seen.

It was likewise impossible to discern any connection between the phenomenon of giant cell formation and behavior and the histologic picture of the nodes transplanted, except that in none of the three sarcomatous nodes were giant cells observable in culture. This was in contrast to fragments of other types of Hodgkin's disease nodes, such as the granuloma and the paragraneloma, from some of which giant cells emerged and from others of which they did not emerge.

Nodes from persons on whom several biopsies were done at different times showed diversity of behavior *in vitro*, even though the histologic aspects remained the same. Thus, in cultures from the first node of 1 patient there were few giant cells, and these few were small and had few nuclei; in cultures of the second node, however, giant cells were much more numerous, were larger and contained more nuclei. The same variation of behavior occurred between nodes of first and second biopsies of another patient; six months later, when cultures were prepared from a third biopsy specimen, no giant cells appeared.

#### COMMENT

The multinuclear giant cells observed in tissue cultures of lymph nodes involved in Hodgkin's disease do not appear to be Sternberg-Reed cells but rather cells of the foreign body type. They occur *in vitro* in cultures of tuberculous nodes, lymphosarcomatous nodes and nodes affected by nonspecific adenitis. They occur also in cultures of buffy coats of centrifuged blood of normal human beings and lower animals and in cultures of spleen and nodes of both human beings and lower animals. These observations suggest the universality of the giant cell in question and emphasize the ease with which it evolves in tissue culture.

In cultures of Hodgkin's disease nodes giant cells appear with such frequency and in such profusion as compared with those seen in cultures of non-Hodgkin's disease nodes that for Hodgkin's disease they may be indicative of some property or process peculiar to the disease. We doubt that the giant cell stimulus arises from the necrotic debris resulting from disintegration of lymphocytes and other cells, because there were many instances of widespread necrosis without giant cells having appeared. Nor can we agree that the giant cells arise as reactants against the coverslip on which the fragment is planted, for control material grown under identical conditions failed to produce reactions comparable to those seen in growing fragments of Hodgkin's disease nodes. For the same reason, too, we could not relate the occurrence of giant cells to the thickness of the clotted medium, nor to the size of the fragment. It would appear, therefore, that the substance responsible for evolution of the giant cells must be produced by the constituents of the tissue fragment. More work will have to be done to identify this substance. Evidence that it may be a virus is still weak; more plausible is the explanation that the giant cells arise in response to some metabolite originating in the tissue grown *in vitro*. Emphasis should be laid on "in vitro" metabolism, because the multinuclear giant cells in question were only rarely found in tissue sections of the original Hodgkin's disease nodes. Finally, the postulate that the giant-cell-stimulating substance is in any way related to the specific cause of Hodgkin's disease, namely, a virus, as suggested by Grand is highly speculative and remains to be established and clarified.

## SUMMARY

Multinucleated giant cells appear in cultures of explants from lymph nodes affected by many nonrelated diseases, likewise in cultures from fragments of spleen, and in cultures of blood cells from the buffy coats of centrifuged blood of both human beings and animals

These cells are of the foreign body type and arise independently of the Sternberg-Reed cell. However, they occur in cultures of a greater number of nodes from Hodgkin's disease sources, in more fragments, in larger numbers, and are larger and have more nuclei, than do similar cells appearing in cultures of nodes from non-Hodgkin's disease sources. The giant cells in question, so conspicuous in tissue culture, are only rarely seen in histologic preparations of the source tissue.

\* These observations suggest that there is present in nodes affected by Hodgkin's disease a peculiar but still unknown factor which is made manifest in tissue culture by the advent of particularly large numbers of multinucleated giant cells of characteristically large size.

## EXPERIMENTAL CORONARY SCLEROSIS

### II The Role of Infection in Coronary Sclerosis of Cockerels

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IN THE first paper in this series<sup>1</sup> a high incidence of "spontaneous" coronary sclerosis in White Leghorn and Barred Rock cockerels was reported. It was shown that the disease was initiated by a focus of degeneration and inflammation of the medial coat of the artery. Intimal proliferation occurred at the point of medial disease as a secondary reaction, and it was markedly accelerated when cholesterol was fed to the birds. The possible causes of the primary medial lesion were discussed, and it was suggested that bacteria or viruses, or allergenic products thereof, might be the agents.

The present report is concerned with the results of experiments which seem to eliminate infection of an acquired type as a cause of the primary lesion.

#### MATERIAL AND METHOD

The first experiment was designed to determine whether or not quarantine against communicable diseases would affect the incidence of coronary sclerosis in cockerels. Sixty 1 day old male White Leghorn chicks were obtained from the Dominion Experimental Farm, Ottawa, and were treated as follows.

Twenty chicks (group 1) were quarantined against infection. They were fed a standard starting ration until they were 3½ months old, and thereafter a standard growing ration to which 2 per cent cholesterol was added. All of the birds survived to the age of 7 months. They were then killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Twenty chicks (group 2) were reared under ordinary laboratory conditions, without quarantine. They were fed the same type of starting ration and the same type of growing ration with added cholesterol as the birds in group 1.

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1 Paterson, J C, Slinger, S J, and Gartley, K G. Arch Path 45:306, 1948.



Nineteen of them survived to the age of 7 months. Then they were killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Twenty chicks (group 3) were reared in quarantine as were those in group 1, and were fed rations identical to those given to birds in groups 1 and 2 except that cholesterol was not added. Thirteen birds survived to the age of 7 months. Then they were killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Additional chicks (group 4), which were of various ages and breeds and each of which was less than 6 months of age at the start of the experiment, were obtained from the Dominion Experimental Farm, Ottawa and were reared under ordinary laboratory conditions. These birds were fed the same diets as were those of group 3 (i.e., without added cholesterol). This group was included to obtain a general picture of the incidence of "spontaneous" coronary sclerosis of cockerels in the Ottawa district.

The rations used in this experiment were supplied by the Department of Poultry Husbandry of the Ontario Agricultural College, and they were identical in their constitution to those described in the first paper in this series.<sup>1</sup> However, before they were used, they were sterilized by autoclaving for fifteen minutes at 15 pounds' (6.5 Kg.) pressure. It was found that this form of sterilization destroyed some of the vitamins, and the deficiencies were made up by feeding unsterilized newly mown grass.

The method of quarantining and protecting groups 1 and 3 against introduction of an infectious agent was that usually followed in a virus unit and consisted in isolating the birds in special rooms and feeding them sterilized foods. In addition, the attendants wore rubber clothing, boots and gloves and had to pass through shower baths to reach the quarantine rooms, and there used a surgical mask.

The incidence and the degree of coronary sclerosis in individual birds were determined by procedures identical to those described in the first paper.<sup>1</sup> Serial sections were cut through the upper three fifths of each heart at intervals of 400 microns. With this technic, from 25 to 30 cross sections of the major coronary system, each showing at least three main arteries, were obtained from each specimen.

The second experiment was designed to determine whether staphylococcic toxin or living staphylococci of a strain virulent to chickens would initiate or accelerate medial lesions in the coronary arteries of chickens.

The staphylococcic toxin was obtained from the Connaught Laboratories, University of Toronto, and it was labeled "24 MA, L.H. Potency = 0.045." It was injected intravenously into 4 white leghorn cockerels, each 6 months old, in doses varying from 0.5 to 2.0 cc. The injections were given five times at intervals of from three to four days. These 4 cockerels, together with three control birds of the same age, were killed from four to seven weeks after the first dose of toxin. Their hearts were sectioned serially, and the intoxicated and the control birds were compared with regard to the incidence and the degree of their arterial lesions.

The living staphylococci had been cultured one year previously from a joint cavity of a white leghorn cockerel which had died during an outbreak of staphylococcic septicemia in our experimental colony. This outbreak of staphylococcosis has been described in a previous communication,<sup>1</sup> and it was considered as a possible cause of the widespread coronary artery disease in the surviving birds. The staphylococci in question were passed through a rabbit in an attempt to enhance their virulence, and the viable material was then injected intravenously.

into 12 white leghorn cockerels, each 2 months old. A single dose, varying from 1,000,000 to 100,000,000 organisms, was given to each cockerel. The birds died or were killed from five to twenty-one days after the injections, and their hearts, together with the hearts of 6 controls of the same age, were sectioned serially and examined for evidence of arterial disease.

### OBSERVATIONS

The incidence of coronary sclerosis in the cockerels in our major experiment was found to be the same regardless of whether or not the birds had been quarantined or fed cholesterol. The data concerning each group are given in the table. It will be noted that approximately the same number of arterial lesions was found in quarantined and non-quarantined birds, both with and without cholesterol feeding. The

*Effect of Quarantine on the Incidence and the Degree of Coronary Sclerosis in Cockerels*

| Procedure                   | Birds Examined | Sections of Heart Examined * | Incidence of Coronary Disease | Average Number of Affected Sections in Diseased Birds | Average Degree of Lesions in Affected Birds |
|-----------------------------|----------------|------------------------------|-------------------------------|---|---|
| Quarantine plus cholesterol | 20             | 519                          | 14 of 20 (70%)                | 6   | Moderate                                    |
| Cholesterol only            | 19             | 487                          | 13 of 19 (68%)                | 5   | Moderate                                    |
| Quarantine plus normal diet | 13             | 302                          | 8 of 13 (62%)                 | 6   | Slight                                      |
| Normal diet only †          | 20             | 435                          | 12 of 20 (60%)                | 5   | Slight                                      |

\* Almost every section showed three or more cross sections of major coronary arteries.

† These birds were of various ages and older than the 7 months which was constant for members of the other three groups.

microscopic appearance of the lesions was identical with that described previously.<sup>1</sup> In cockerels on a standard diet the media was the site of hydropic degeneration and round cell infiltration, and the adjacent intima was thickened, vacuolated and rather fibrous. On the other hand, cholesterol-fed birds showed lesions of media and intima which were appreciably larger (but not more numerous), and these points were heavily infiltrated with foam cells.

A virulent strain of staphylococci or of staphylococcal toxin injected intravenously in repeated doses failed to initiate, or to accelerate, the lesions of coronary sclerosis in cockerels under the conditions of our second experiment. The incidence, the distribution and the degree of disease were the same in the treated birds as in the control birds. Individual lesions were encountered in the intoxicated group which were unusually severe (like that shown in the figure), but identical lesions were seen in control birds in the same experiment.

## COMMENT

The present experiments seem to eliminate acquired infection as a cause of coronary sclerosis of chickens. The quarantine technic of our major experiment was admittedly imperfect, it cannot be compared, for example, with that used by Reyniers and associates<sup>2</sup> for rearing small animals under bacteria-free conditions. But, at the same time, it was probably rigid enough to reveal some difference in the incidence of



High power photomicrograph of a small coronary artery of a 6 month old White Leghorn cockerel which had been given intravenously five doses of staphylococcic toxin, of 2 cc each, and killed one month after the first injection. The intima is thickened and vacuolated, and the underlying media shows marked necrosis of muscle fibers. A similar grade of necrotizing mesarteritis was noted in a control bird. Hematoxylin and eosin,  $\times 300$ .

arterial disease in the various groups if acquired infection had been the source. This impression is supported by the results of our subsidiary experiments with staphylococcic infection, which gave entirely negative results.

Attention is therefore being directed elsewhere in attempting to elucidate the cause of coronary sclerosis of chickens. Congenital infection particularly leukosis of fowl, is under consideration, but even more emphasis is being placed on the possibility of hypersensitivity to dietary

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<sup>2</sup> Reyniers, J. A., and others. Rearing Germ-Free Albino Rats, Lobund Report, no 1, University of Notre Dame, South Bend, Ind., 1946.

agents The diets used in the experiments impress one with their extreme artificiality, and these same diets are in general use in the poultry industry today

It is to be noted that cholesterol feeding did not affect the incidence or the distribution of arterial disease in birds in the present experiments, but it did accelerate the progression of individual lesions This potentiating effect of cholesterol feeding on the course of "spontaneous" coronary sclerosis of chickens has already been described,<sup>1</sup> and is here confirmed in chickens bred in a different part of Canada

#### SUMMARY AND CONCLUSIONS

White leghorn cockerels which had been quarantined against infectious disease until they were 7 months old failed to show any reduction of the incidence of coronary sclerosis as compared with that observed in control cockerels which had been reared under natural conditions The intravenous injection of staphylococci of a strain virulent to chickens, and of staphylococcic toxin, failed to produce arterial lesions or to accelerate those of the spontaneous variety The experimental findings suggest that infectious disease of an acquired type is probably not responsible for the medial lesions which initiate coronary sclerosis in white leghorn cockerels

# ROLE OF AGE IN ESTROGEN-INDUCED LYMPHOID TUMORS OF MICE

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AND

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ST LOUIS

THE AGE of the animals at the beginning of the treatment is one of the factors that determine the incidence of estrogen-induced mammary cancers in mice. According to the studies of Loeb,<sup>1</sup> there is in males a maximum susceptibility at about the time of onset of sexual maturity. In an extension of these investigations we<sup>2</sup> noted an age factor operative, though in a limited way, in estrogen-induced leukemia in castrated male mice of strain Dbu. Subsequently, an age factor was shown to play a part in the incidence of leukemia caused by irradiation.<sup>3</sup> More recently, we<sup>4</sup> have studied the role of age in the production of mammary cancer in castrated and noncastrated male mice of strain C3H treated with an estrogen. Again, lymphoid tumors developed in a number of these animals, and it is on these findings and their dependence on age that we wish to report.

## MATERIALS AND METHODS

Sixty-six male mice of strain C3H raised in our laboratory were castrated at the age of 3 to 4 weeks. Twenty-four of these castrates and 26 animals with intact testicles received subcutaneous injections of 0.03 mg of alpha estradiol benzoate once a week.<sup>5</sup> This compound was dissolved in sesame oil and administered for five months beginning at the age of 4 to 5 weeks. Forty-two castrated and 33 noncastrated mice received the same treatment, but the injections were started when the animals had reached the age of 4 months. The mice were caged

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1 Loeb, L. Biol Symposia **11** 197, 1945.

2 Silberberg, M., and Silberberg, R. Proc Soc Exper Biol & Med **58** 347, 1945.

3 Kaplan, H. S. (a) Cancer Research **7** 141, 1947, (b) J Nat Cancer Inst **9** 55, 1948.

4 Silberberg, M., and Silberberg, R. Proc Soc Exper Biol & Med **69** 438, 1948.

5 The Schering Corporation supplied progynon B®.

in groups of 4 or 5 each. They were fed a stock diet of commercial chow and had water available at all times. The mice were examined at regular intervals, and sick-looking ones were killed. At necropsy, mammary glands, pieces of spleen, liver, lymph nodes, heart, lung, the endocrine organs and some bones were removed, fixed in 4 per cent formaldehyde and embedded in paraffin, and the sections were stained with hematoxylin and eosin. Mice that had died were inspected for gross changes, and if autolysis was not too far advanced, the internal organs were likewise taken out for histologic examination.

In this report the term "cancerous lymphoma" will be used to include lymphosarcoma as well as leukemia.

#### OBSERVATIONS

Cancerous lymphoma was not observed in 95 normal males of our C3H stock. Of 178 breeding females 8 to 21 months old, 2 showed

#### *Summary of Experimental Data*

| Group | Experiment   | Total<br>No<br>of<br>Mice | Animals Reaching<br>Lymphoma Age |         |       | Animals in Which Cancerous<br>Lymphoma Developed |                |         |       |
|-------|--|---------------------------|----------------------------------|---------|-------|--|----------------|---------|-------|
|       |  |                           | No                               | Age, Mo |       | No   | Per<br>centage | Age, Mo |       |
|       |  |                           |                                  | Mean    | Range |  |                | Mean    | Range |
| 1     | Noncastrated mice<br>received estrogen<br>from age of 1 mo | 26                        | 19                               | 12.8    | 9-17  | 4  | 21.1           | 15.0    | 14-17 |
| 2     | Castrated mice re-<br>ceived estrogen<br>from age of 1 mo  | 24                        | 19                               | 13.2    | 9-18  | 6  | 31.6           | 13.5    | 9-17  |
| 3     | Noncastrated mice<br>received estrogen<br>from age of 4 mo | 33                        | 14                               | 13.5    | 9-20  | 1  | 7.1            | 18.0    |       |
| 4     | Castrated mice re-<br>ceived estrogen<br>from age of 4 mo  | 42                        | 34                               | 13.9    | 9-19  | 2  | 5.9            | 18.0    | 17-19 |

cancerous lymphoma at the age of 19 months. A few old breeders had moderately enlarged mesenteric lymph nodes.

The results of our experiments are presented in the table. The numbers of mice in which cancerous lymphoma developed in the various experimental groups and the ages at which death occurred are recorded. A correlation between the occurrences of mammary cancer and cancerous lymphoma could not be established. Animals referred to as "reaching the lymphoma age" are those that lived nine months and more, lymphoma not having been observed before the age of 9 months.

*Group 1* (mice with intact testicles receiving estradiol benzoate from the age of 1 month on)—Of a total of 26 animals, 19 reached the lymphoma age. The mean age at death of all animals in this group was 12.8 months, with a range from 9 to 17 months. In 4 of them (21.1 per cent) cancerous lymphoma was found at a mean age of 15 months, the age range being 14 to 17 months.

*Group 2* (mice castrated at the age of 3 to 4 weeks and treated with estradiol benzoate from the age of 1 month on)—Of 24 animals 19 lived

to reach the lymphoma age. The mean age at death was 13.2 months, with a range from 9 to 18 months. In 6 of these mice (31.6 per cent) cancerous lymphoma developed, and they either died or were killed at a mean age of 13.5 months. The youngest animal with cancerous lymphoma in this series was 9 months old, the oldest 17 months old.

*Group 3* (mice with intact testicles receiving estradiol benzoate from the age of 4 months on) —Of 33 mice, 14 reached the lymphoma age, the mean age at death being 13.5 months, with a range from 9 to 20 months.<sup>6</sup> One animal (7.1 per cent) died with cancerous lymphoma at the age of 18 months.

*Group 4* (mice castrated at the age of 3 to 4 weeks and treated with estradiol benzoate from the age of 4 months on) —Of 42 mice, 34 reached the lymphoma age. The mean age of these animals at death was 13.9 months, with a range from 9 to 19 months. In 2 of these mice (5.9 per cent) cancerous lymphoma appeared at the age of 17 and 19 months, respectively.

In the experimental mice the diagnosis of cancerous lymphoma could be made after examination of the gross specimen in most cases. In some animals soft, whitish mediastinal tumors originating in the thymus were seen filling a large part of the thoracic cavity, in others similar neoplasms were found in the mesentery or attached to the intestine. The cervical, mediastinal, mammary and para-aortic lymph nodes and the liver were invariably enlarged, the spleen was from four to six times the usual size, and firm and granular on the cut surface. Liver and kidney showed an ochre brown color, and the lungs were pale. In estrogen-treated animals with or without cancerous lymphoma an oblong, moderately firm, reddish mass, varying in size from about 7 by 3 by 3 mm to 15 by 5 by 5 mm, was frequently noted in the mesentery near the pancreas.

#### HISTOLOGIC OBSERVATIONS

The macroscopic diagnosis could readily be confirmed in microscopic sections. The usual structure of the lymph nodes, and to a varying degree that of the spleen, was obliterated by diffusely invading cancerous cells of the lymphocytic series. Liver, kidney, lungs and heart likewise showed numerous areas of this cellular infiltration (figs 1 to 3). However, the involvement of the various organs and the size of the individual foci differed considerably. In the liver the earliest changes consisted of

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<sup>6</sup> The high mortality of these animals was due to the frequent occurrence of calculosis of the urinary bladder and advanced pyelonephritis. Many of these mice died suddenly, without previous evidence of illness, and the organs were too autolyzed to permit adequate histologic examination. Owing to the small number of animals available for study, the results do not lend themselves readily to statistical evaluation. However, in view of the fact that the findings were similar to those in the following group they are probably significant.

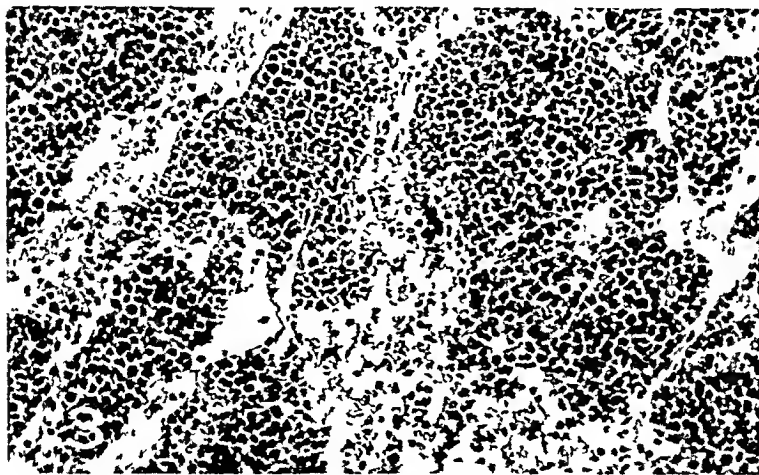
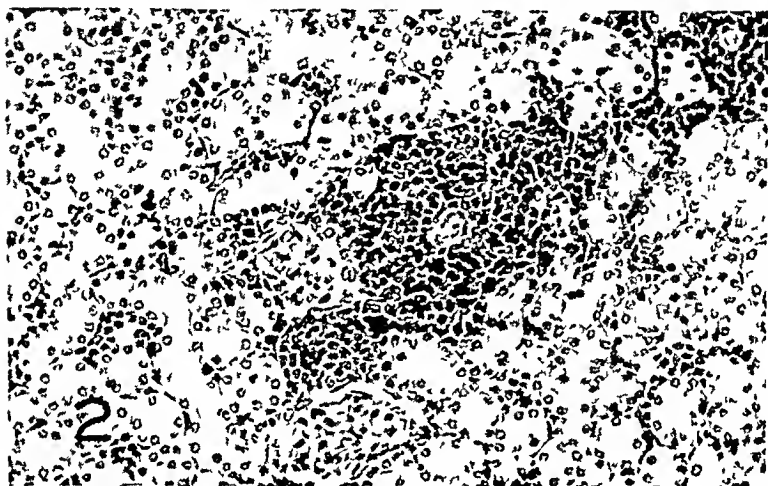
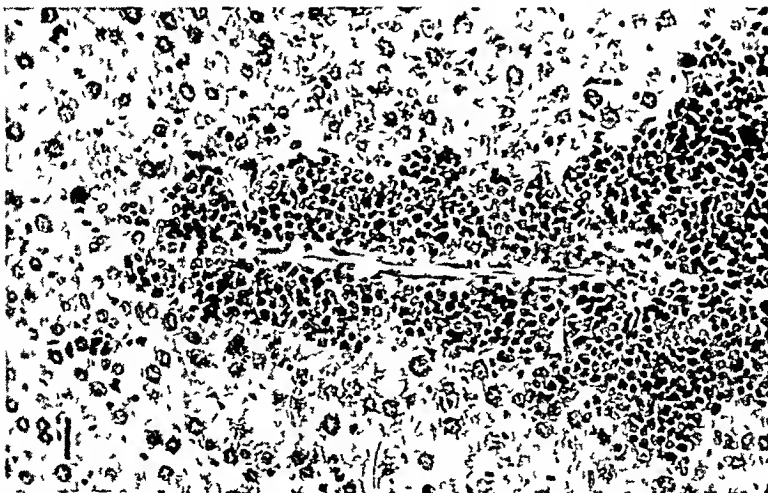


Fig 1—Leukemic infiltration of the liver of a 14 month old castrated male mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on,  $\times 180$

Fig 2—Cancerous lymphoma of the kidney of a 9½ month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on,  $\times 180$

Fig 3—Cancerous lymphoma of a mesenteric lymph node of the mouse whose renal involvement is shown in figure 2,  $\times 180$



widely scattered small perivascular foci composed of a few immature blood cells, some of which showed mitotic figures. Megakaryocytes were seen here and there. In more advanced cases, the cellular infiltration was more extensive, and more organs were affected. In leukemic animals, immature white blood cells were seen in the sinusoids of the liver and in the blood vessels. The cancer cells were usually of the

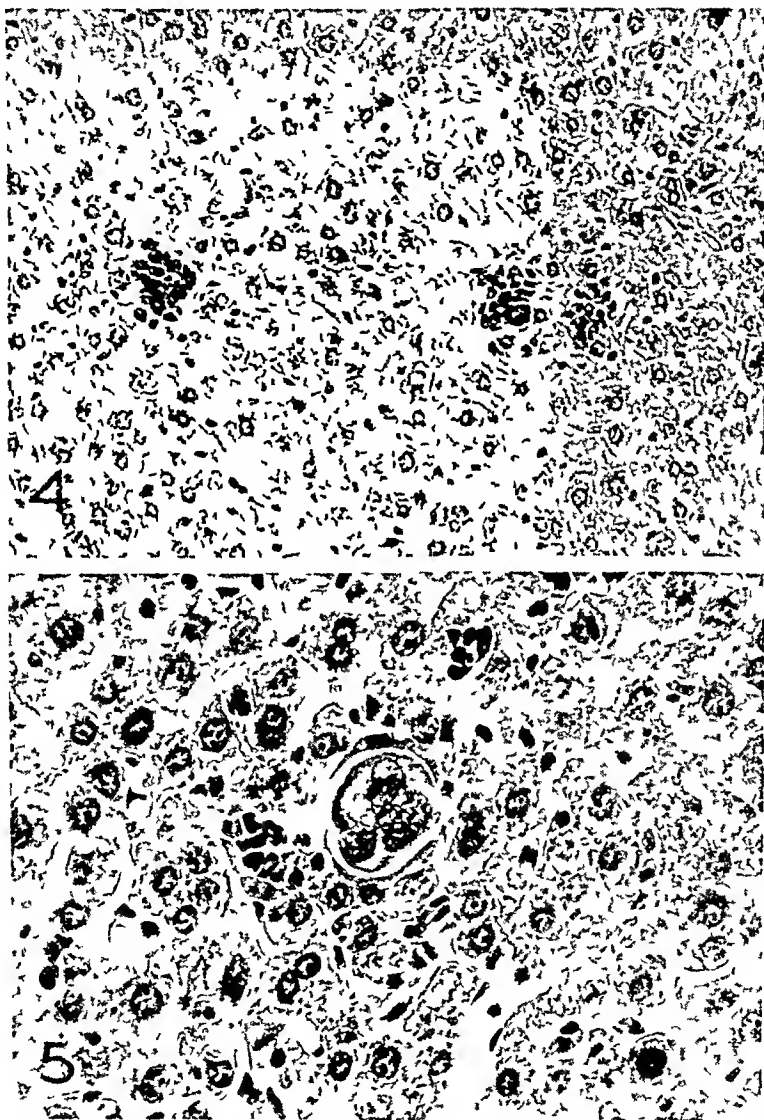


Fig 4—Extramedullary hemopoiesis in the liver of a 16 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months,  $\times 180$

Fig 5—Focus of extramedullary hemopoiesis in the liver of a 14 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months  $\times 360$

erythrocytic series (rúbriblasts) or the lymphocytic series. Only once was myelogenous leukemia found with a variety of myeloid cells infiltrating the tissues.

In about one third of the animals, hemopoiesis was found in spleen, lymph nodes, kidney and liver (figs 4 and 5). The hemopoietic foci were small and scattered through these organs, they consisted predominantly of cells of the granulocytic and erythrocytic series and megakaryocytes.

The mesenteric mass was unrelated to cancerous lymphoma, since it occurred not only in animals in which lymphoma had developed but in those in which this tumor had not been observed. The mass was composed of normal or cancerous lymphatic tissue with dilated, engorged sinuses of varying size and distribution (figs 6 and 7). These structures are apparently identical with those observed by Gardner and co-workers<sup>7</sup> in mice of the same strain. In some animals there was a diffuse but moderate dilatation of the sinuses, in others the engorgement was more localized, and the individual spaces had reached considerable size. Laminated thrombi were frequently present in the lumens (fig 8). The lining endothelium was regular throughout and showed no evidence of proliferation. Thus, in agreement with the view of Gardner,<sup>7</sup> these formations are not to be considered as neoplastic. Whether they represent an analogue to the hypervolemia of the liver occurring after transplantation of granulosa cell tumors<sup>8</sup> remains to be decided.

#### COMMENT

In the following paragraphs the present findings will be discussed and compared with earlier results obtained in similar experiments in male mice of strain DbA.

In males of strain C3H receiving injections of estradiol benzoate from the age of 1 month on, castration raised the incidence of cancerous lymphoma from 21.1 to 31.6 per cent. In corresponding males of strain DbA the incidence of lymphoma was 30 per cent in the noncastrates and 50 per cent in the castrates. In strain C3H the mean age at death of mice with cancerous lymphoma was 15 months in the noncastrates and 13.5 months in the castrates, in strain DbA the mean age at death of lymphoma was 11.6 months in the noncastrates and 11.3 months in the castrates.

In mice of strain C3H orchidectomized at 3 to 4 weeks of age and given injections of estradiol benzoate from the age of 4 months on, the

<sup>7</sup> Gardner, W. U., Dougherty, T. F., and Williams, W. L. *Cancer Research* **4** 73, 1944.

<sup>8</sup> Furth, J., and Sobel, H. (a) *J. Nat. Cancer Inst.* **7** 103, 1946, (b) *Science* **105** 41, 1947. Gardner<sup>7</sup>.

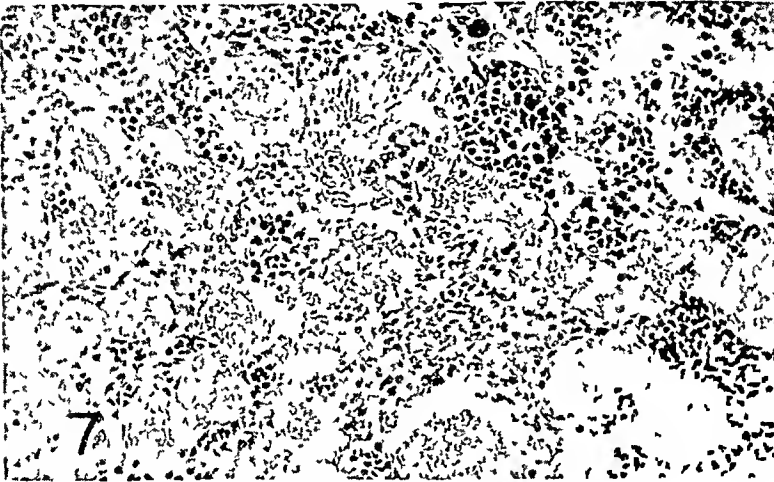


Fig 6—Enlarged sinuses of a mesenteric lymph node of a 16 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months,  $\times 95$

Fig 7—Diffuse enlargement of sinuses of a mesenteric lymph node of a 13 month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 4 months on,  $\times 180$

Fig 8—Large thrombus in a dilated sinus of mesenteric lymph node of a 17 month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on,  $\times 95$

incidence of lymphoma was 5.9 per cent, it was 7.1 per cent in the non-castrated mice of this age group. The mean age at death was 18 months for both the castrated and the noncastrated animals. The estrogen administered to mice of strain DbA had in the older age group produced cancerous lymphoma in 43.5 per cent of both the castrated and the non-castrated mice, the mean age at death being 11.6 months in the former and 12.9 months in the latter.

A discrepancy seems to exist as regards the incidence of cancerous lymphoma in the older groups of noncastrates of strain C3H and DbA. In noncastrates of strain C3H treated from the age of 1 month on, the incidence of lymphoma was 21.1 per cent, in those treated from the age of 4 months on, it was 7.1. In corresponding groups of DbA males this decline was not observed. On the contrary, the younger series showed a 30 per cent and the older one a 43.5 per cent incidence of lymphoma. There was, however, a difference of about ten days in the age at which castration was performed and estrogen treatment was begun in the two strains. The mice of strain DbA were about 3 weeks and those of strain C3H about 4½ weeks old at the beginning of the treatment. This change in the procedure was necessary because of the high mortality of our young C3H males. While an age difference of ten days seems small, it may suffice to influence the results of the experiments as shown by Loeb and co-workers<sup>9</sup> in experiments on the role of age in estrogen-induced breast cancer. The tumor incidence of mice receiving estrogen from the age of 2 weeks was lower than that of mice treated from the age of 4 weeks on. Similar conditions have recently been observed in regard to lymphoma induced by irradiation.<sup>3</sup> As in the aforementioned experiments,<sup>9</sup> a maximum susceptibility to estrogen induction of lymphoma may occur at or about sexual maturity. Our C3H mice were apparently close to this stage, whereas our DbA mice might not yet have reached the peak of susceptibility. In strain DbA a slight increase in the susceptibility would have sufficed to raise the incidence of cancerous lymphoma from the 30 per cent in the younger group to or above the 43.5 per cent found in the older series.

These findings allow several conclusions. (1) In young males of both strains the testicle exerts an inhibiting effect on the production of cancerous lymphoma by estrogen if treatment is begun at 3 to 5 weeks of age, (2) in neither strain does the absence of the testicle change the incidence or the time of appearance of cancerous lymphoma if the estrogen treatment is started three months after castration. The role played by the testicle in estrogen induction of cancerous lymphoma in one of the two strains used is thus similar to its role in the other.

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<sup>9</sup> Loeb, L., Sontzeff, V., Burns, E. L., and Schenken, I. R. Arch. Path. 38: 52, 1944. Loeb<sup>1</sup>

However, differences were observed with regard to the influence of the age factor in experimental leukemogenesis. In castrates of strain C3H treated at an early age cancerous lymphoma was about five times as frequent (31.6 per cent) as in the castrates treated from the age of 4 months on (5.9 per cent). Therefore in strain C3H an extratesticular factor aids in determining the incidence of cancerous lymphoma produced by estrogen. This factor may reside within the hemopoietic tissues themselves and may consist of decreasing responsiveness to leukemogenic stimuli with advancing age. The aging hemopoietic tissue of males of this strain would thus act as does the mammary gland in response to administration of estrogen.

In contrast to conditions in strain C3H the influence of age, if any, on estrogen induction of cancerous lymphoma in strain DbA is of minor significance. Fifty per cent of the castrates given injections from the age of  $\frac{1}{2}$  month on and 43.5 per cent of those treated from the age of 4 months on showed cancerous lymphoma. This strain difference in regard to the role of the age factor may possibly be connected with the difference susceptibility of the two strains to estrogen induction of lymphoma.<sup>10</sup> In strain C3H, the incidence of estrogen-induced cancerous lymphoma was lower in all groups, and the mean age at death (13.5 to 18 months) was higher than in strain DbA (11.1 to 12.9 months). This indicates a lesser susceptibility of strain C3H as compared with that of our strain DbA. The more marked the susceptibility to exogenous stimulation—or the more powerful the stimulus—the less significant may become the role in leukemogenesis of secondary factors, such as age, and perhaps even that of the testicles. In the highly susceptible strain DbA neither the removal of the testicles nor the age of the animals noticeably affected the incidence and time of appearance of cancerous lymphoma as they did in strain C3H. In the latter strain, by contrast, the lower susceptibility might have permitted the relatively weak secondary factors to assert themselves and to exert a more obvious influence on the incidence of cancerous lymphoma. Whether this suggestion might apply also to estrogen-induced mammary cancers remains to be decided. Our findings on the significance of age in the development of estrogen-induced breast cancers of C3H males would support this point of view.<sup>4</sup> In castrated males of strain C3H receiving estradiol benzoate from the age of 1 month on, the incidence of mammary cancers was 44.4 per cent, it was 30 per cent in castrates given injections from the age of 4 months on. Thus the incidence of mammary cancer dropped only slightly (about 33 per cent) as the age at the beginning of the estrogen treatment advanced. These same groups of males, however, showed an incidence of lymphoma of

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10 Kirschbaum, A. *Yale J Biol & Med* **17** 163, 1944

31.6 and 5.9 per cent respectively. The age difference in the two groups thus caused an impressive (about 400 per cent) decrease in the incidence of cancerous lymphoma in the older group. Therefore, the age factor exerted a more obvious influence on estrogen induction of leukemia than on estrogen induction of mammary cancer. Since strain C3H is more susceptible to the development of cancer of the breast than to that of cancerous lymphoma, the role of the age factor seems to be inversely proportional to the degree of susceptibility.

#### SUMMARY

The susceptibility of male mice of strain C3H to estrogen induction of cancerous lymphoma varied with the age at which the administration of the estrogen (alpha estradiol benzoate) was begun. A maximum susceptibility was found about the onset of sexual maturity. Castration performed at the age of 3 to 4 weeks raised the incidence of cancerous lymphoma if the treatment with estradiol benzoate was started one week after orchidectomy. Castration failed to influence the incidence of cancerous lymphoma if the estrogen treatment was begun at the age of 4 months. In castrates receiving the estrogen from the age of 4 months on, the incidence of cancerous lymphoma was significantly lower than in castrates treated with estradiol benzoate from the age of 1 month on. Thus an extratesticular age factor, presumably residing in the aging hemopoietic tissues, aids in determining the outcome of this type of leukemogenesis. The significance of the age factor varies in different strains and seems to decrease as the genetic susceptibility of the animals to estrogen induction of cancerous lymphoma increases.

# DEVELOPMENT OF ANAL DUCTS AND GLANDS

With Reference to the Pathogenesis of Anorectal Disease

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IT HAS been assumed by certain investigators that the anal crypts and contiguous ducts and glands provide a route by which infection is transmitted to the deeper tissues of the anal region. The anal crypts, ducts and glands have thus been considered as a factor in the genesis of anal fissures, abscesses and fistulas. The possibility that the ducts of the anal glands may become obstructed or that the distal portion of uncanalized glands may have secretory activity could easily explain the development of cysts and of those abscesses and fistulas which do not communicate with the anal canal.

In a previous study of the anal ducts and glands Hill, Shryock and ReBell<sup>1</sup> observed that these structures varied widely in number and in the depth to which they penetrated into the submucosal and muscular tissues. These observations were based on 17 specimens from a 7 month fetus, 5 newborn infants and 11 adults. It was observed that in some specimens from newborn infants the ducts lacked complete canalization and that in 1 of the adult specimens there was a simple retention cyst of the tubular part of an anal gland. It was reported that smooth muscle occurred uniformly in the submucosa in adult specimens, but that in specimens from newborn infants the smooth muscle was merely in the process of differentiation.

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1 Hill, R, Shryock, E H, and ReBell, F G J A M A 121 742, 1943

Although that study supported the concept that the anal glands play a definite role in the genesis of anorectal disease, it also raised several questions, the answers to which were dependent on a detailed study of the embryonic development of the anal glands. Previous studies of the development of these glands have been based on a small number of specimens and have therefore not permitted accurate conclusions with respect to the number of anal glands present in the various fetal age groups.

The present study was undertaken, therefore, in the hope of finding answers to the following questions: Do anal glands tend to grow deeper throughout the stages of fetal development? What percentage of anal glands penetrate the muscularis? Do the anal glands canalize progressively? Does an obstructed gland or duct represent one which has not yet canalized or one which has become secondarily obstructed? What is the time relation between the differentiation of the muscularis mucosae and the development of the anal glands? When does the "muscularis submucosae ani" develop?

#### REVIEW OF THE LITERATURE

Johnson<sup>2</sup> (1914) was the first to publish accurate information with respect to the detailed structure and location of the anal glands. His study was based on serial sections and reconstructed scale models. In his review of the literature, Johnson acknowledged the earlier contributions of Herrman and Desfosses<sup>3</sup> (1880) and of Herrmann<sup>4</sup> (1880), who were presumably the first workers to differentiate the anal glands (intramuscular glands) from the anal crypts and from the circumanal glands. Herrmann and Desfosses observed that some of the anal glands penetrated the internal sphincter. Herrmann<sup>4</sup> (1880) was unable to find any trace of secretory epithelium in these "special acinous glands" or intramuscular glands and therefore admitted that they might be simply sinuses rather than true glands.

Braun<sup>5</sup> (1901) was unable to find the anal glands described by Herrmann and therefore completely denied their presence. Johnson<sup>2</sup> stated that these tubular structures resembled glands and were lined with 2 to 3 layer cuboidal epithelium, but he found no evidence that the epithelium was glandular in character. However, Hill, Shryock and ReBell<sup>1</sup> (1943) presented evidence in their study that at least some portions of these structures were definitely glandular. They were able to demonstrate cells showing apical cytoplasmic vacuoles and found that these cells stained for mucin with Krajian's carbofuchsin method.

2 Johnson, F. P. *Am J Anat* **16** 1, 1914

3 Herrmann, G., and Desfosses, L. *Compt rend Acad d sc* **90** 1301, 1880, cited by Johnson<sup>2</sup>

4 Herrmann, G. *J de l'anat et de physiol* **16** 434, 1880, cited by Johnson<sup>2</sup>

5 Braun, W. O. *Untersuchungen über das Tegument der Analöffnung* Inaug. Dissert., Königsberg, R. Leupold, 1901, pp 1-50, cited by Johnson<sup>2</sup>



Tucker and Hellwig<sup>6</sup> (1935) also referred to these structures as glandular ducts and stated that they had observed them developing from the upper portion of the zona intermedia in the 30 mm embryo

Bremer<sup>7</sup> (1936) stated that the secretory cells of the anal ducts disappeared in postnatal life, but Hill and associates<sup>1</sup> presented photographs of a cyst of an anal gland from an adult which implied that secretory activity had persisted

Questions have been raised as to the direction of penetration of the glands Johnson<sup>2</sup> stated that they may extend either cephalad or caudad This was confirmed by Hill and his group, who found that most of the glands penetrated laterally and caudad but that a few extended in a cephalad direction The study of Kratzer and Dockerty<sup>8</sup> (1947), however, did not add confirmatory evidence Their study of serial sections of an 8 month stillborn boy indicated that there was no penetration upward Most writers agree that lateral penetration may extend to or through the internal sphincter

A variation in the degree of canalization of the intramuscular glands was noted by Johnson<sup>2</sup> and by Hill, Shryock and ReBell<sup>1</sup>

Harris<sup>9</sup> (1929) emphasized that certain of the intramuscular glands penetrated deeply and reached the inner circular layer before the muscularis mucosae had differentiated This is apparently substantiated by the findings of Johnson<sup>2</sup> and Tucker and Hellwig<sup>6</sup> The latter referred to the glandular ducts which were present in the 30 mm embryo Johnson stated that in the 190 mm embryo the muscularis mucosae was absent from the anal region but that at birth it was completely formed and was prolonged in several strands which extended down into the rectal columns

Fine and Lawes<sup>10</sup> (1940) called attention to the constant presence of muscularis submucosae and in the region of the pecten band The deepest of these smooth muscle fibers were in close contact with the internal sphincter, while the superficial ones were said to be continuous with the muscularis mucosae of the rectum The fibers were sometimes scattered and sometimes gathered into definite muscle bundles All the specimens investigated were from adults

The present study of the anal canal of the fetus provides answers for some of the questions, hitherto unanswered, concerning the development of these various structures

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6 Tucker, C C, and Hellwig, C A Arch Surg 31 521, 1935

7 Bremer, J L A Textbook of Histology Arranged upon an Embryological Basis, ed 5, Philadelphia, P Blakiston's Son & Co, 1936, p 296

8 Kratzer, G L, and Dockerty, M B Surg, Gynec & Obst 84 333, 1947

9 Harris, H A Proc Roy Soc Med 22 1331, 1929

10 Fine, J, and Lawes, C H W Brit J Surg 27 723, 1940

## MATERIALS AND METHODS

A microscopic study was made of the anal canals of 49 fetuses. These fetuses ranged in crown-heel length from 9.5 to 38 cm. Serial sections were made of each anal canal. All sections were stained in a routine manner with hematoxylin and eosin.

Scale models were made of 4 selected specimens from 12 cm, 17.5 cm, 23 cm and 30.5 cm fetuses. The models were constructed by the laminated cardboard technic, serial sections were projected and traced on cardboard, and the tracings were then cut out and assembled in a serial sequence.

## OBSERVATIONS

Anal glands were found in 26 (53 per cent) of the 49 fetuses studied. No glands were found in specimens from fetuses less than 16 cm in length (about four months' gestation).

The glands varied with respect to depth of penetration (fig. 1). Some extended only to the submucosa, others, to the inner circular layer of muscle, and the deepest ones were found in the external longitudinal layer of muscle. Seventy-seven per cent of all the glands seen penetrated into the muscularis.

The number of glandular elements per specimen varied from 1 to as many as 13. The average number of glands and/or ducts in the submucosa only was 3.7 per specimen. The average number in the muscularis was 5.4.

The reconstruction of the specimen from the 30.5 cm fetus clearly showed the complex branching of the terminal portions of some of the glands (fig. 2). It was also evident from the model that some of the terminal branches extended in a cephalad direction.

The question of canalization of the glands was investigated. Four specimens presented one uncanalized gland and 1 showed two uncanalized glands. Canalization does not necessarily progress peripherally. In 1 case (fig. 3) a gland was demonstrated to be uncanalized proximally and distally but well canalized in its more central part. Surrounding the uncanalized segments there was no evidence of inflammation.

All glands were found to be connected with the anal canal.

The muscularis mucosae had not differentiated in fetuses under 16 cm in length. It was quite constantly present in fetuses above 24 cm but was not well differentiated in all specimens.

## GENERAL CONSIDERATION

The observations incident to the present study suggest that the anal glands are not easily distinguished until about the fourth month of intrauterine life. Even in cases in which differentiation may have been initiated at an earlier age, there is no penetration of the muscularis until about the fourth month.

In view of the earlier report<sup>6</sup> of Tucker and Hellwig it was surprising to find that no glands occurred in the 10 specimens from fetuses under

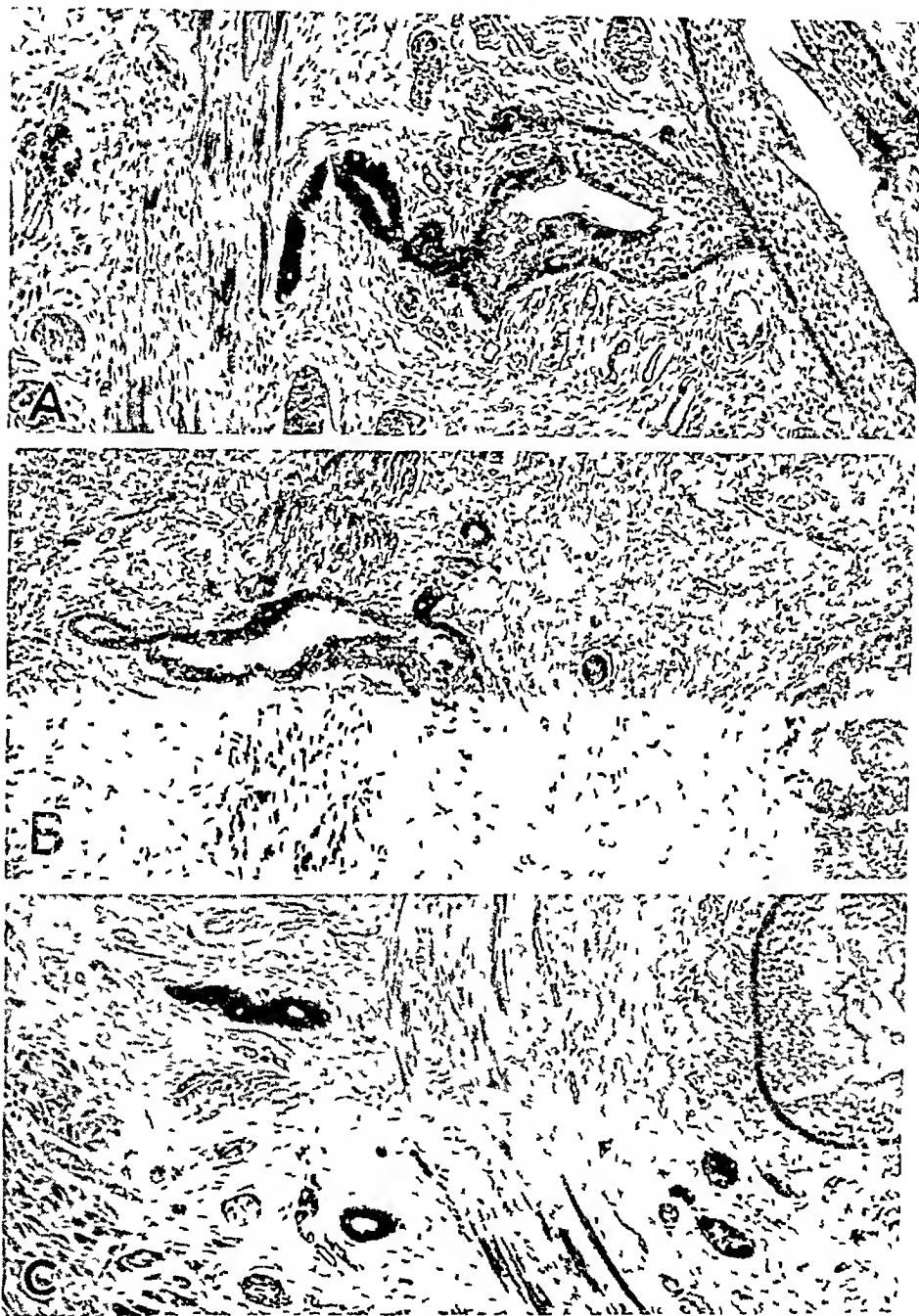


Fig 1—Anal glands penetrating to varying depths A, into the submucosa ( $\times 100$ ), B, through the circular muscle layer ( $\times 70$ ), C, into the longitudinal muscle layer ( $\times 70$ ) Hematoxylin and eosin stain

16 cm in length This finding was borne out not only by microscopic study but also by the reconstructed model of the specimen from the 12 cm fetus

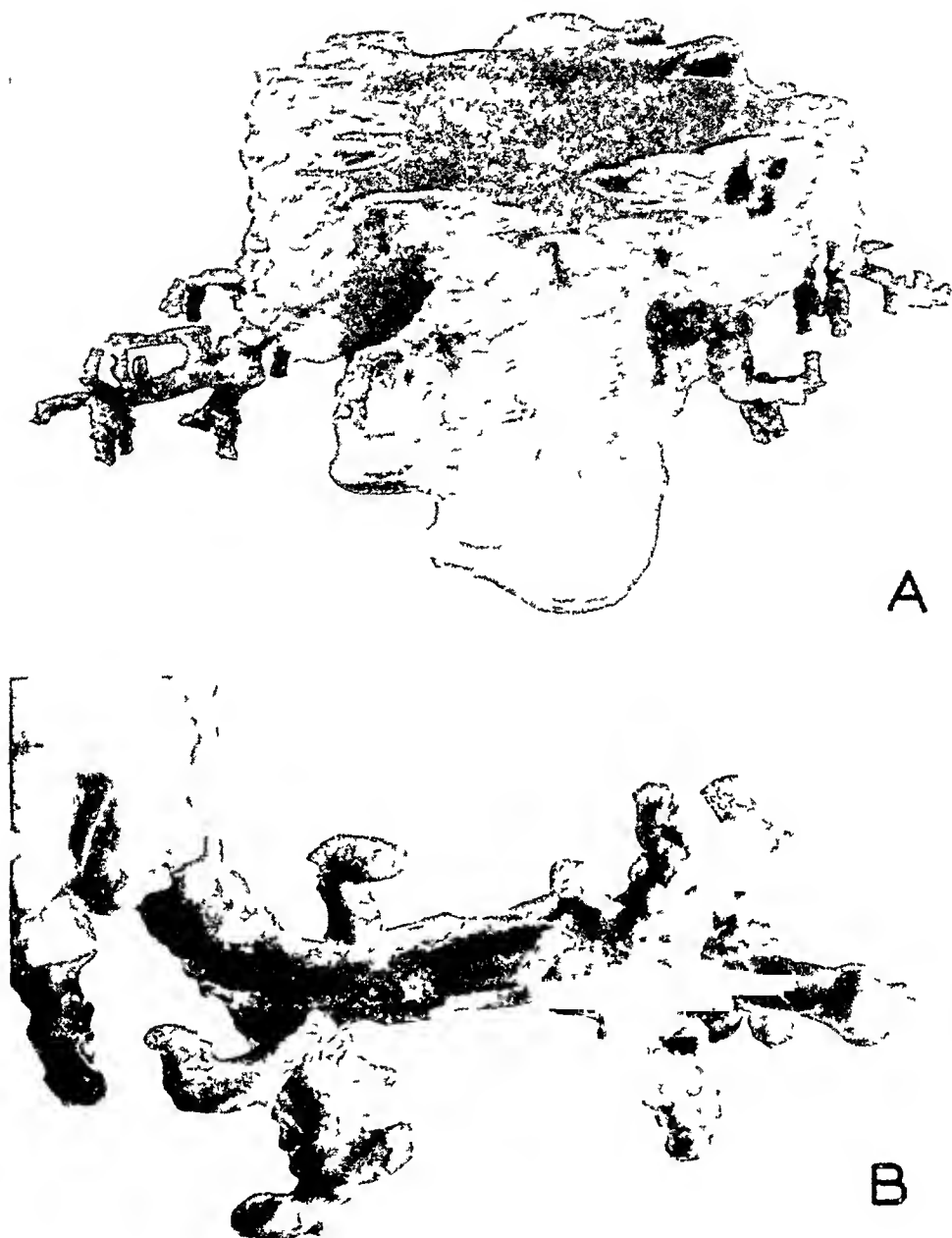


Fig 2—*A*, reconstruction of the anorectal region from a 30.5 cm fetus showing numerous glands and their complex branching, *B*, single glandular element (enlarged) of the anorectal region from a 30.5 cm fetus showing branching cephalad as well as caudad

The anal canal of the fetus presents a number of longitudinal folds, which may be so complex in contour as to be confused with rudimentary anal ducts or glands. Positive identification requires the sequential

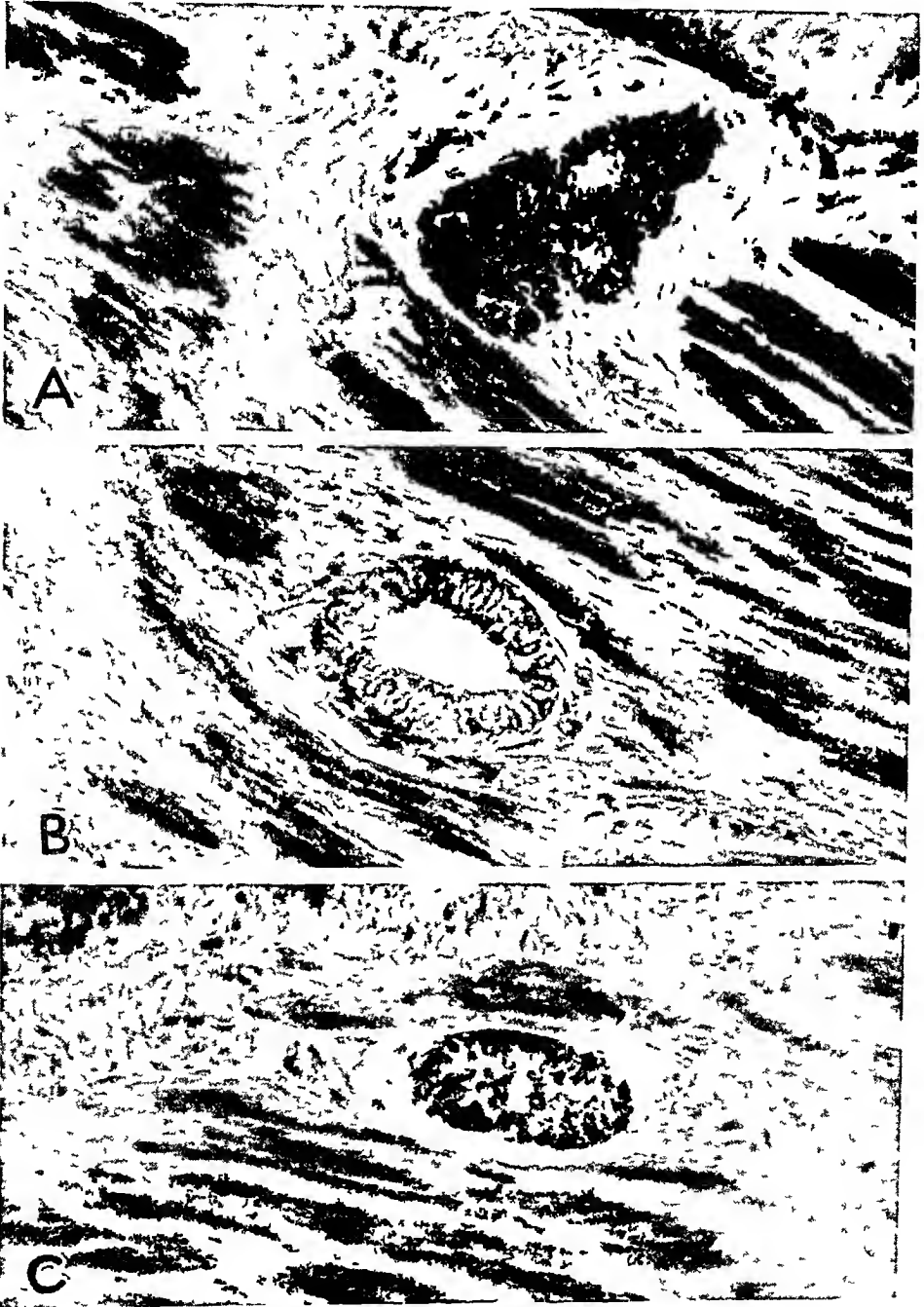


Fig 3—Sections through an anal gland showing incomplete canalization. *A*, proximal closed portion ( $\times 270$ ), *B*, middle open portion ( $\times 270$ ), *C*, distal closed portion ( $\times 270$ ). Hematoxylin and eosin stain.

study of serial sections and preferably recourse to a scale model. In several of the specimens the mucosa was folded in such a way as to

resemble, in a single microscopic preparation, the developing ducts which Tucker and Hellwig observed in their specimen from a 30 mm fetus. Detailed study of the serial sections indicated, however, that those features were only complex folds and not ducts or glands. It must be admitted that in specimens in which the glands penetrate but a short distance, as into the submucosa only, it is difficult to be sure whether a given structure is a duct or a simple fold, for the epithelium lining a confirmed duct may appear much like that lining the zona intermedia of the anal canal. Differentiation becomes simple, however, when a gland is seen penetrating the muscularis.

It was interesting to note that so many (77 per cent) of all the glands seen penetrated into the muscularis. This study did not suggest any correlation between fetal age and depth of gland penetration. It has been suggested that the ducts and glands form avenues for the spread of infection. This deep penetration seems to give a reasonable explanation of certain rectal and perirectal lesions.

Certain of the terminal branches of the glands were found to penetrate cephalad as well as caudad (fig 2), thus confirming the earlier work of Johnson<sup>2</sup> and of Hill, Shryock and ReBell<sup>1</sup>. If these cephalad branches become infected, sinus tracts result which may extend variable distances up the wall of the rectum. These may again communicate with the lumen and thus establish complete fistulas.

Embryologically, all the glands are epithelial outgrowths of the anal mucosa, but because of incomplete canalization a limited number may never actually communicate with the anal canal. Thus it is possible that a gland such as shown in figure 3 might develop into a cyst if the proximal portion remained uncanalized.

The observation that some specimens do not have glands and that those which do may show considerable variation in the number of glands present is important from the standpoint of the spread of infection. Those persons with a large number of glands are more susceptible than are those with few or no glands. It would seem, therefore, that a certain percentage of persons are predisposed to perirectal and perianal complications by reason of the development of intramuscular glands in the anorectal region.

A search was made for muscle fibers in the submucosa which might conform to the description given by Fine and Lawes<sup>10</sup> of what they termed muscularis submucosae ani. Hill and associates<sup>1</sup> stated that in the newborn infant the smooth muscle of the submucosa of the anal canal is in a process of differentiation, muscle cytoplasm being obvious only in scattered areas. In the present series we did not find anything that we thought could be called muscularis submucosae ani. None of the specimens were from fetuses larger than 38 cm, so one would infer that the muscularis submucosae ani must differentiate later in fetal life.

In our study it became evident that the muscularis mucosae does not differentiate at any definite age. It was present in a specimen from a 16 cm fetus and not in a specimen from a 35 cm fetus. However, it apparently never differentiates before the third or fourth month of intra-uterine life. In view of this one cannot state positively that the glands develop before the muscularis mucosae or vice versa. However, the poor differentiation of the muscularis mucosae in the early fetal specimens suggests that it usually develops later.

#### SUMMARY AND CONCLUSIONS

A microscopic study has been made of the anal canals of 49 fetuses ranging in crown-heel length from 9.5 to 38 cm. Laminated models were made of selected specimens. Photographs of models and microscopic sections are presented herewith.

Particular attention was given to the time of differentiation of the anal glands, the muscularis submucosae and the muscularis mucosae. The depth and the direction of penetration, as well as the canalization, of the glands were investigated.

Anal glands were found in 53 per cent of the specimens studied. None were found in specimens from fetuses under 16 cm long. In the majority of instances the glands had penetrated the muscularis, thus providing an avenue by which infection might spread to deeper layers of the anorectal tissues.

The number of anal glands present in a single specimen varied from 1 to 13. It is believed that those persons who have a relatively large number of glandular elements have a greater predisposition to perianal and perirectal pathologic conditions, which probably are due to infection entering and spreading through these channels.

Six incompletely canalized glands were found. If the proximal portions remain uncanalized, the distal parts may develop into cysts, which predispose to abscess and fistula formations having no communication with the anorectal canal.

The muscularis submucosae and was not identified as a definite structure in any specimen of this series. Presumably, it develops after the seventh month of intrauterine life.

The muscularis mucosae does not develop prior to the third or the fourth month of intrauterine life, and even as late as the seventh month it may be poorly differentiated.

#### DISCUSSION

DR C. C. TUCKER, Wichita, Kan. It has become established that anal ducts play an important role in infection arising in and around the anal-rectal line. In the past twelve years the number of specimens which my associates and I have examined microscopically have run into the thousands. Only in rare instances

have we found that these ducts were not infected. The infections observed ranged from the chronic to the acute stage. We have also noted that the pathologic process is confined to ducts which open into the crypts of Morgagni, that the ducts are natural incubators for the colon bacillus, the staphylococcus, the gonococcus, the streptococcus and, in rare instances, the tubercle bacillus. That these ducts are preformed structures and not results of an ulcerative process was demonstrated by us on dissecting specimens without inflammatory changes. We found these ducts sometimes extending into the internal sphincter muscle. They are either simple tubular ducts or the more complex branching structure which extends from the mucosa of the anal canal into the submucosa or the muscular layer.

The invading bacteria pass through the crypts of Morgagni into the ducts and attack the epithelial cells lining the lower parts of the ducts, the epithelium changes to columnar cells. The crypts themselves are lined by stratified squamous cells which are much more resistant to bacterial infection except in gonococcal infection of the female. Because of the anatomic structure of the anus, the parts become bathed in the gonorrheal discharge at the time of defecation, thus the crypts of Morgagni become infected as well as the ducts. In fistulas, we believe, the origin of infection is confined to the anal ducts.

Drs. Hill, Small, Hunt and Richards are to be commended on the work they have done on the embryonic development of anal ducts and glands.

Time and space will not permit me to go into the embryologic phase of this paper, but I hope that in the future I shall have the privilege of doing so.

DR J. P. NESSELROD, Evanston, Ill. My associates and I became interested in this problem of the role played in anal infection by the anal glands and ducts. Our work differs in this way. The anatomic material is adult and not fetal. Therefore we are more likely to find, as we did in our work at Northwestern University, considerable round cell infiltration of the tissues that are involved. Contrary to the observations of Hill and his associates I was unable to demonstrate, except in an occasional instance, glandular invasion of the muscular structures. Whether this is due to the difference in our anatomic material I do not know. We may not agree exactly in our ideas of these glands, but I believe we are driving at the same thing and that is anal infection and its role in the pathogenesis of anorectal inflammatory disease. By anal infection is meant the sum total of events taking place in the development or the pathogenesis of anorectal inflammatory disease, and I include not only those entities mentioned by Dr. Hill and his associates but also hemorrhoids.

This work of Hill and his associates affords another striking demonstration of the contribution made by the basic sciences to the everyday work of the clinician and the surgeon. This concept of anal infection and its role in anorectal inflammatory disease marks a prominent step forward in understanding of these problems. Only at such time as the practicing clinician becomes aware of this process will he be able to give his patient the proper advice. We will then relegate the so-called shortcut methods to their proper place as compared with adequate surgical management.

DR EDWARD LEVY, New York. All can congratulate the authors for a masterly presentation. In my own anatomic studies I came across a paper submitted by a German for the doctor's degree about 1867, in which he described an unusual or abnormal form of crypts, and in the illustrations accompanying the paper he showed crypts about the size of those which I have seen in the microscopic slides of Dr. Hill's demonstration at the exhibition. Following that, one waited until about 1914, when Johnson did his work on the embryonic anorectal canal, and for the first time a reconstruction of intramuscular crypts was demonstrated. There



is available now, in Baltimore, Johnson's reconstruction of glands, which he observed in the embryo, penetrating for a variable distance from the crypts into the deep tissue

About 1937 Dr Tucker presented before the American Proctological Society in Atlantic City a paper by Tucker and Hellwig integrating this anatomic finding with clinical findings. I believe from that moment history was made.

The work of Hill and his associates, as presented at this meeting and their scientific exhibit are a confirmation of the work of Johnson. This work is a classic contribution to proctologic literature.

From now forward consideration of clinical disease of the anorectal area must include an evaluation of the anal ducts and glands. I believe that history was made in proctology this morning.

DR MALCOLM R HILL, Los Angeles. Dr Tucker confined his discussion to the anal crypts and ducts. To us, in our investigation, on two occasions, anal crypts and ducts appeared to carry with them contiguous gland elements which in many instances gave evidence of secretory function which was demonstrable on proper staining.

Dr Nesselrod's study, which has been confined to pathologic material, is interesting. I have tried to save representative tissue in operative cases to pass on to the pathologist. Proctologic sampling of the anal canal in light of proper conservation of normal continuity of tissues does not give the complete clinical pathologic picture. Dr Nesselrod has linked the causation of hemorrhoids with infection of these microscopic elements.

## HISTOPATHOLOGIC OBSERVATIONS IN A FATAL CASE OF Q FEVER

THEODORE L. PERRIN, M D  
BETHESDA, MD

ONLY 6 fatal cases of Q fever are on record<sup>1</sup>. Autopsies were performed in 4 of the 6 cases, 2 were reported in the "Annual Report on the Health and Medical Services of the State of Queensland, Australia"<sup>1a,b</sup> 1 by Lillie, Perrin and Armstrong<sup>1c</sup> and 1 by Brown, Knight and Jellison<sup>1e</sup>. The purpose of the present report is to describe the histopathologic observations in the last of these cases<sup>1e</sup>. The clinical and gross postmortem findings and the procedures by which the virus was isolated have been presented in detail by Brown and co-workers<sup>1e</sup>.

### REPORT OF A CASE

A 43 year old white man died after an illness of fifteen days. During the first week, the symptoms were those of a "head cold," and the patient was able to work at his regular occupation as a cattle handler in a stockyard. During the second week he became acutely ill. Prominent clinical findings were cough, nasal congestion, chills, profuse perspiration, frontal headaches and mental confusion. The temperature was elevated, varying between 103 and 106 F. Q fever was not suspected, and a diagnosis of influenza complicated by pneumonia was made. Therapy included the administration of penicillin in oil, sulfadiazine and oxygen, and was ineffectual. An examination of the blood three days before death produced normal values for erythrocytes, hemoglobin and color index. However, there was slight leukopenia, the total white blood corpuscle count was 5,000 per cubic millimeter, and the differential count was 74 neutrophilic granulocytes, 25 lymphocytes and 1 eosinophilic granulocyte per hundred cells. The patient died suddenly after a convulsive seizure.

*Gross Postmortem Findings* (forty hours after death) —The body had been embalmed by arterial injection only. Pertinent findings were limited to the thorax. Excess fluid was noted in the pleural and pericardial cavities, and all the lung tissue appeared to be consolidated except for a small area in the upper lobe of the right lung. The cut surfaces of the lung tissue were wet, and

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1 (a) Annual Report on the Health and Medical Services of the State of Queensland, 1936-1937, vol 22, (b) 1938-1939, vol 52 (c) Lillie, R D, Perrin, T L, and Armstrong, C. Pub Health Rep 56 149, 1941 (d) Irons, J V, and Hooper, J M. J A M A 133 815, 1947 (e) Brown, D C, Knight, L A, and Jellison W L. California Med 69 200, 1948

no purulent material was seen. A firm fibrinous clot, about 8 cm in length, was found in the branch of the pulmonary artery leading to the upper lobe of the left lung. The gross postmortem diagnoses were atypical pneumonia and pulmonary embolism.

*Isolation of Virus*—In guinea pigs inoculated with blood drawn three hours after death and with sternal marrow obtained at autopsy symptoms consistent with Q fever developed, and complement fixation tests of their serums were positive for Q fever in dilution of 1:64 or greater. Rickettsias were demonstrated in smears from some of the inoculated animals.

*Microscopic Observations*—Heart. Sections of the anterior wall of the left ventricle revealed marked patchy fibrosis of the myocardium and moderate to marked arteriosclerosis of the anterior descending branch of the left coronary artery. The lumen of one large collateral of the descending branch was completely obliterated by an old, organized thrombus. In a section from a localized area of thinning near the apex of the left ventricle the muscle fibers had been almost completely replaced by dense fibrous tissue, which contained scattered small accumulations of hemosiderin.

Lungs. Sections from the areas of gross consolidation revealed a confluent pneumonic process. The exudate was moderately abundant in many areas, but in scattered foci it was scanty. Frankly hemorrhagic exudate was encountered in some alveoli, but for the most part erythrocytes were inconspicuous, and numerous large mononuclear cells were intermingled with fewer neutrophilic granulocytes and occasional lymphocytes. Some of the large mononuclear cells were of the macrophage type and were actively phagocytic, while others exhibited large round to oval leptochromatic and trachychromatic nuclei and relatively scanty basophilic cytoplasm. Large mononuclear cells of the latter type often lined portions of interalveolar septums. A loose fibrin network was often encountered in the exudate, and occasionally the fibrin was condensed at the periphery of an alveolus or an alveolar duct, forming an indistinct hyaline membrane. Inter-alveolar septums were focally thickened and irregularly congested, and hyaline thrombi were sometimes seen in septal capillaries. The small bronchi and bronchioles contained exudate similar to that seen in the alveoli, and partial desquamation of mucosal epithelium was occasionally noted. In a few bronchi portions of denuded mucosa were covered by a single layer of flattened, deeply stained epithelial cells. Medium and large bronchi seldom contained exudate and were not involved by the inflammatory process. Small numbers of large mononuclear cells and lymphocytes were seen in peribronchial and perivascular connective tissue and were present in moderate numbers focally in the pleura and in edematous interlobular septums.

Sections from areas of the lung which were least involved grossly showed moderate congestion, patchy edema, focal hemorrhage, and small to moderate numbers of hemosiderin-laden macrophages in many alveoli. Scattered small pneumonic areas were present, and although neutrophilic granulocytes predominated in many of these foci, large mononuclear cells and lymphocytes were also present, and the large mononuclear cells were often numerous. Lymphocyte and mononuclear cell infiltration of peribronchial and perivascular connective tissue was more marked than that occurring in the areas of confluent pneumonia and was often associated with similar infiltration of thickened interalveolar septums.

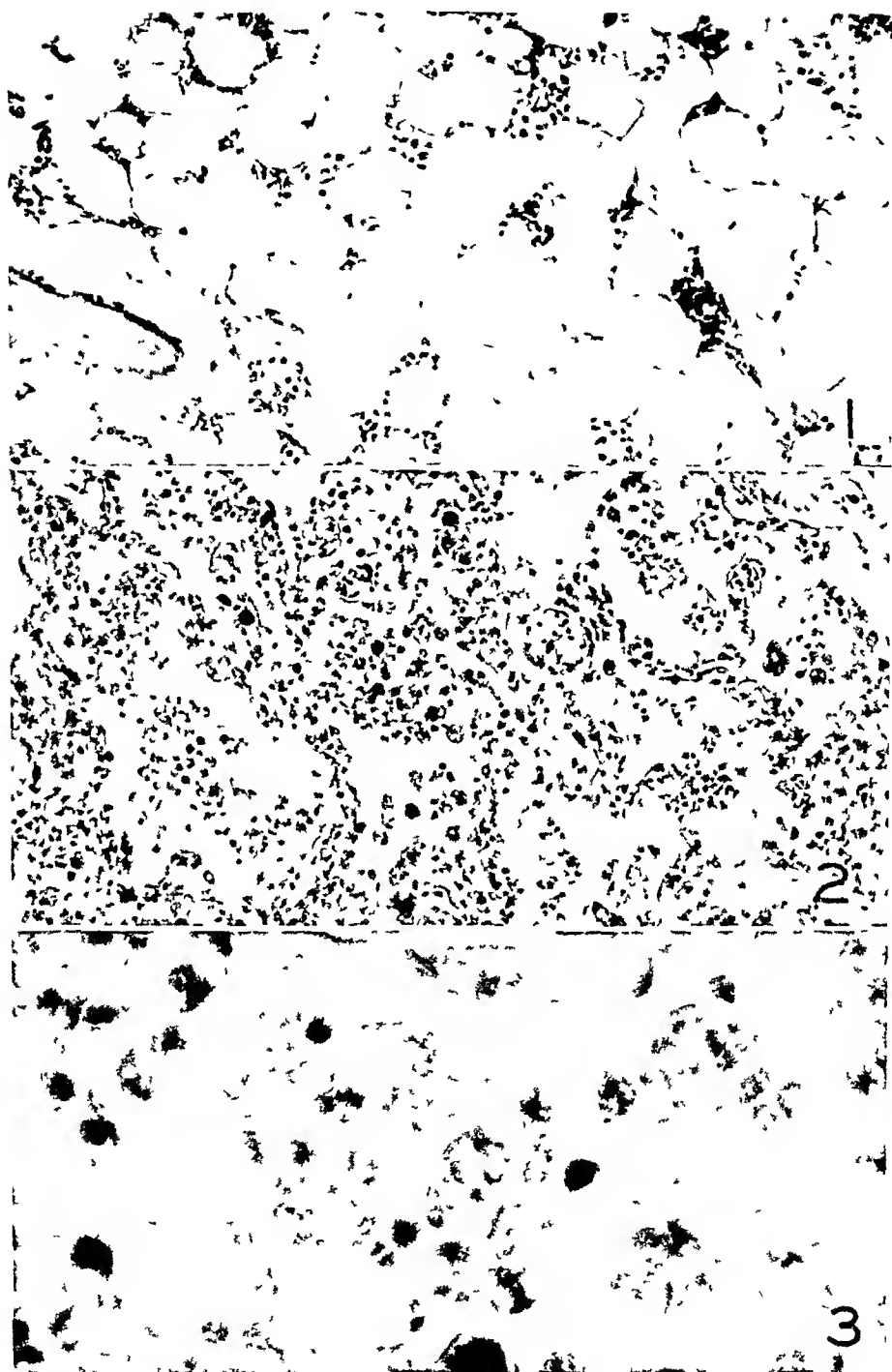


Fig 1—Area of hypoplasia in sternal marrow  $\times 225$

Fig 2—Thickening of interalveolar septums Note alveolar exudate showing predominance of mononuclear cells  $\times 225$

Fig 3—Swollen alveolar lining cells Note mononuclear cells in the alveolar exudate  $\times 860$

A careful search through several sections stained with the azure-eosin technique<sup>2</sup> and Gram's stain failed to reveal rickettsias or other micro-organisms

Sections of blood clot taken from a branch of the pulmonary artery leading to the upper lobe of the left lung were consistent in appearance with postmortem blood clot

Liver The organ revealed no abnormality

Spleen Plasmacytes and large mononuclear cells were found in slightly increased numbers in the red pulp

Lymph Nodes A peribronchial lymph node exhibited considerable deposition of carbon In a mesenteric lymph node, widely dilated sinuses contained a moderate number of large mononuclear cells

Kidneys There was slight to moderate fibrous intimal thickening of the small and medium-sized renal arteries

Adrenal Glands The adrenal glands showed no abnormality

Sternal Marrow In a few patchy areas the marrow was almost, if not completely, devoid of myeloid cells In these areas a little precipitated albuminous fluid was sometimes seen, and occasional small accumulations of hemosiderin The remaining, major portion of the marrow revealed the normal proportion of myeloid cells and fat In the cellular areas, erythropoiesis was within normal limits, and megakaryocytes were present in usual numbers However, mature granulocytes were relatively few, and the majority of cells were classed as myelocytes and metamyelocytes

The histopathologic diagnoses were bronchopneumonia, focal hypoplasia of bone marrow with a moderate left shift of cells of the granulocytic series, moderate coronary arteriosclerosis, with an old, organized occlusive thrombus of a major collateral of the left anterior descending branch of the coronary artery old, healed infarction of the anterior wall of the left ventricle, slight renal arteriosclerosis, congestion of spleen

#### COMMENT

In the case now described there is some evidence to suggest that the bronchopneumonia and the hypoplasia of marrow may have been due to the direct action of the causative agent of Q fever The lesions of other organs were considered to be either nonspecific or degenerative in type The Q fever virus was isolated from blood and marrow and satisfactorily identified, and bacteria could not be demonstrated in appropriately stained sections of lung tissue The bronchopneumonia with mononuclear cells predominating in the exudate was consistent in type with a rickettsial infection and resembled the bronchopneumonia in another fatal case of Q fever<sup>1c</sup> In the other 2 cases of Q fever in which autopsies were made,<sup>1a b</sup> histopathologic observations were not recorded, and a comparison of lesions cannot be made In experimental Q fever infections of mice,<sup>3</sup> guinea pigs<sup>4</sup> and monkeys<sup>1c</sup>

2 Lillie, R D Histopathologic Technic, Philadelphia, The Blakiston Company, 1948, p 82

3 Perrin, T L, and Bengston, I A Pub Health Rep 57 790, 1942

4 Lillie, R D Pub Health Rep 57 296, 1942

bronchopneumonia was frequent and, as noted in 2 human cases, mononuclear cells predominated in the exudate. However, in the experimental infections there were lesions in many other organs and tissues. Foci of hypoplastic marrow were found only in the human case described in this report and in the experimentally infected mice. Rickettsias were not demonstrated in tissue sections from any of the human patients, nor in those from the monkeys or the guinea pigs. They were seen in sections from the mice only after several serial mouse to mouse passages of the virus.

The available evidence is insufficient definitely to implicate the virus of Q fever as the etiologic agent responsible for the major pathologic changes in the case here presented. Many additional cases must come to autopsy and be carefully studied before the pathology of human Q fever can be established. In such cases, adequate bacteriologic examinations should supplement and control virus isolation procedures because only limited reliance can be placed on the demonstration that microorganisms are or are not present in tissue sections, and rickettsial lesions cannot be differentiated from certain bacterial lesions on the basis of histopathology alone.

As a final point of interest, attention is directed to the fact that important nonrickettsial lesions were encountered in 3 of the fatal human cases in which autopsies were made. A complete record of the post-mortem observations was not included in the report of the fourth case.<sup>1b</sup> In 1 of the 3 cases widespread bilateral pulmonary tuberculosis was observed,<sup>1a</sup> in another cardiac enlargement and dilatation with a widened mitral orifice,<sup>1c</sup> and in the present case marked coronary arteriosclerosis with an old, organized myocardial infarct. It is suggested that these lesions probably exerted an unfavorable influence on the course of the disease. This is in accord with the clinical observation that deaths from Q fever are rare in otherwise healthy persons.

#### SUMMARY

The sixth recorded fatal human case of Q fever is that of a 43 year old man who died after an influenza-like illness lasting fifteen days. Significant lesions encountered on histopathologic examination included bronchopneumonia characterized by a predominantly mononuclear cell exudate, focal hypoplasia of the bone marrow with a moderate left shift of cells of the granulocytic series, and moderate to marked coronary arteriosclerosis with an old, organized myocardial infarct. When the lesions were compared with those reported in other fatal human cases and with experimental infections it appeared that the bronchopneumonia and the lesions of the marrow may have been due to the infectious agent of Q fever. It is suggested that the cardiac condition may have exerted an unfavorable influence on the course of the disease.

## THE MOLE AS A POSSIBLE RESERVOIR OF POLIOMYELITIS

L E RECTOR, M D

ST LOUIS

IF A SINGLE animal or insect is ever found to be the reservoir of poliomyelitis, serving as a host or a vector, one may anticipate (1) that it will be found throughout the temperate and tropical zones, (2) that it will account for the frequently observed tendency of the disease to start and to have a higher incidence in rural than in urban populations, (3) that it will account for the seasonal tendency of the disease in man, and (4) that it will be so inconspicuous as to have escaped consideration to date. Numerous animals have been considered, many of them being more or less susceptible to the virus after adaptation, yet none so far has merited serious consideration as the natural reservoir, and only in the simians has the disease been observed to occur naturally. After considering the fossorial habits and the geographic distribution of the ground mole one is struck with the possibility of this animal fulfilling the four anticipated features of a natural host for poliomyelitis.

The mole is found in practically every country in the temperate and tropical zones, even being found in Siberia<sup>1</sup>. Only islands such as Ireland<sup>2</sup> and islands of the Pacific Ocean such as the Hawaiian group,<sup>3</sup> which have arisen geologically separated from the continental mainlands, are free from moles.

It is obvious that a higher percentage of the rural than of the urban population comes in contact with these animals, yet the prevalence of moles in the city parks offers urban dwellers sufficient contact with them—disregarding trips to the country. Furthermore, spot maps of urban epidemics of poliomyelitis frequently show a centripetal spread.

That moles come out on the surface of the ground during the hot summer months in search of worms and water is a well established fact among naturalists,<sup>2</sup> and even the untrained naturalist is impressed by the observation that in hot weather their runways are so shallow that they are covered merely by the roots of overlying vegetation. Coupled with these observations is the even more important one that during hot weather people lie on the ground, frequently in close contact

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From the Department of Anatomy, Washington University

1 Skalon, W N. *Zool Anz* **77** 307, 1928

2 MacDougall, R S. *Tr Highland Agric Soc Scotland* **14** 80, 1942

3 Hamre, C J. Personal communication to the author

with mole runs. Furthermore, in cold weather moles use their deeper runs and refuse to burrow new runways when the ground is frozen. In fact, the mole is vermivorous, and the depth of his burrow will vary seasonally with the level at which his food, chiefly the earthworm, is to be found. Earthworms breed in the hot summer months and are known to come to the surface and draw leaves into their burrows for nesting purposes. The mole is voracious, with its eating said to be a frenzy, and it will incur many dangers to get food.

Numerous animals and many insects have been studied as a possible reservoir for the virus of poliomyelitis, but as yet the mole has escaped consideration. This is probably due to the fact that his subterranean existence leaves him somewhat inconspicuous. However, this same subterranean existence could easily facilitate an inherently inconspicuous harboring of the virus between the seasons of common epidemic occurrence of the disease in man. Were the animal to be found susceptible to the virus one could easily conceive of epizootics occurring undetected by man. If the disease were found to be highly fatal to them, they would die and bury the virus with them, only to pass it on to another mole when the latter eventually broke into and took over the run left idle by the dead mole. It is known that the virus of poliomyelitis when kept in a moist state and in darkness will remain viable at room temperature, for periods varying from 25 to 114 days.<sup>4</sup> In the natural environment of the mole, the requirements of darkness and moisture would be maintained, and the temperature probably would be lower than room temperature. Furthermore, if the disease should be less fatal among moles and leave surviving but partially paralyzed animals, one would have to consider whether there are carrier states, how long the virus is discharged in the stools and pharyngeal secretions and whether dissemination occurs during the mating season (March and April).

It is now well established that the virus of poliomyelitis can be recovered from the pharyngeal secretions for a few days and from the stools for several weeks following the onset of human infection. Also the virus has been demonstrated in sewage coming from epidemic areas on at least eight occasions.<sup>5</sup> Should the mole be found susceptible to the virus, the possibility of an epizootic starting with exposure to human excreta is likely.

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4 Landsteiner, K., and Levaditi, C. *Ann Inst Pasteur* **24** 833, 1910. Kling, C., Levaditi, C. and Lepine, P. *Bull Acad de med, Paris* **102** 158, 1929.

5 (a) Paul, J. R., and Trask, J. D. *Am J Pub Health* **32** 233, 1942. (b) Toomey, J. A., Takacs, W. S., and Weaver, H. M. *Am J Dis Child* **70** 293, 1945.



In any event one is faced with the irrefutable fact that man and mole come much closer to each other during the hot summer months, and it seemed advisable to investigate the mole's susceptibility to the virus of poliomyelitis

#### MATERIALS AND METHODS

All moles were caught manually in Forest Park in St Louis. An attempt to keep a stock bin of animals was soon abandoned because of the tendency of moles to fight until there is one surviving victor when more than one is placed in the same container. Consequently each mole was kept in an individual metal can that was partially filled with dirt. After moles 6, 7, 8 and 9 became paralyzed, probably from being exposed to contaminated dirt, new dirt was used for each animal, and used dirt was decontaminated with cresol solution before being discarded. A container of water was kept countersunk to the level of the surface of the dirt. All moles except mole 36 were fed diced horse meat or ground beef or both. Mole 36 was fed solely on earthworms. Feeding was effected by merely placing the meat on the surface of the dirt. Rectal temperatures were taken daily after inoculation or exposure and more frequently if the animal was being observed while showing symptoms. The isolation technic was employed in handling the animals, the hands and arms being washed with soap and water and rinsed in alcohol before handling, and between, moles. In addition, the cotton glove worn on the hand used to hold the mole while its temperature was being taken was boiled from 5 to 10 minutes between moles.

The experiment was begun by inoculating moles 1 and 2 intracerebrally with a filtrate of a mouse brain infected by a rodent-adapted strain of Lansing virus. A second but similarly infected mouse brain was the source of the inoculums used for moles 3, 4 and 5, a third similar mouse brain was used as the source of inoculums for moles 33, 34, 35 and 36. The human brain and spinal cord used as inoculum for the animals indicated in table 1 were obtained at the autopsy of an 11 year old boy who died in a respirator in the summer of 1946 with a classic clinical and pathologic picture of poliomyelitis. This virus had been preserved in water and glycerin at ordinary ice box temperature, and the particular strain of virus involved is unknown.

Stools from 4 children currently stricken with poliomyelitis (summer of 1948) served as the source of inoculums or of soil contamination for moles 37 to 43, inclusive. Clinically these children showed paralysis and an abnormality of the spinal fluid consistent with poliomyelitis.

Each inoculation passage from mole to mole, from mole to Swiss mouse or cotton rat, and between mouse and rat was made with a saline dilution (approximately 1:10 to 1:20) of a filtrate from wet brain and spinal cord. All filtrates were made by passing the material through a Chamberland L-5 filter except the stool filtrates injected into moles 40, 41, 42 and 43, for which the stools were passed through a Berkefeld N candle. The filtrates of the latter were in 1:1 dilution.

Prior to inoculation moles 24 through 29, inclusive, were subjected to a period of starvation varying from 24 to 27 hours with the hope that the hypoglycemic state might enhance their susceptibility to the virus as Sandler<sup>6</sup> found it did for rabbits.

No animals were killed, all being observed until they died. Autopsies were performed as soon after death as practicable. In many instances moles died during

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6 Sandler, B. P. *Am J Path* 17:69, 1941

the night, and in other instances the animals were placed under refrigeration for varying periods. Brain and spinal cord saved for subsequent passage were preserved in the fresh state in dry ice (solid carbon dioxide). Tissues studied microscopically were fixed in acetic acid-Zenker's solution and stained with hematoxylin and eosin.

#### EXPERIMENTAL RESULTS

*Two Types of Terminal Picture*—One type of terminal picture encountered, and the one most classically representing a poliomyelitic attack, was that of massive paralysis of the abductor group of muscles of the forelegs. In its most complete form there was no motion of the anterior extremities, and the animals invariably were found on the surface, usually on their backs, unable to turn over. They frequently would lie in this position for 24 to 36 hours with the forelegs inactive while the hindlegs kept up a continuous running motion. When these animals were turned over to a normal running position, their forelegs were adducted, motionless, resembling sled runners as the hindlegs frantically propelled the body. This type of activity continued until death. Starvation and dehydration are undoubtedly important factors in the failure of these animals to recover.

Slight variations of this picture were noted. In some cases only one foreleg demonstrated paralysis. This resulted in the animal's turning in circles, with the affected side serving as the axis. In others there was transient paralysis, with fairly good function returning later. In the case of mole 10, at one time the hindlegs were paralyzed, and propulsion was effected by use of the forelegs. Paralysis of the hindlegs was definitely observed in 4 cases and questionably in another, compared with 13 definite and 3 questionable cases of involvement of the forelegs. Animals judged to show questionable involvement of the forelegs were so classified because they made frantic attempts to dig into the dirt when disturbed but did not appear to have the strength to do so.

The other type of terminal picture observed was that in which the animal stayed on top of the dirt, "huddled up," with its neck flexed and its head held against its chest. Animals so affected frequently showed instability of equilibrium, falling from side to side. If sufficiently stimulated, they would move around, frequently burrowing into the dirt, only to return to the surface in a few minutes to resume their former position. Definite ability to move the extremities was demonstrable. Respirations were usually labored at first and shallow terminally. Unfortunately, the mole seems normally to employ chiefly an abdominal type of respiration. The picture closely resembles what has been previously referred to as a vagal type of poliomyelitis in experimental animals.

In 1916 Rosenau and Havens<sup>7</sup> described a fulminating type of death observed in rabbits inoculated with poliomyelitis virus. The symptoms were explosive in character, and death usually occurred within a few hours to 2 days.<sup>\*</sup> The death of mole 19 in this series fits this picture, and a similar type of death may have occurred in moles dying during the night.

*Survival of Animals*—No animals were killed, and of the 43 moles used in the experiment only 2 still survive (moles 40 and 43). Several of the moles may have died as the result of inoculation trauma or other causes, this is especially true of moles 24, 25, 27, 28 and 29, which were subjected to a period of starvation prior to and subsequent to inoculation. It is doubtful if the mole can survive more than 36 hours of starvation. All animals have been included in table 1 for completeness.

7 Rosenau, M. J., and Havens, L. C. J. Exper. Med. 23: 461, 1916.

TABLE 1—Data on Moles Undergoing Experiments

| Mole | Days in Captivity Before Inoculation | Route of Inoculation * | Volume of Inoculum (Cc), Amount of Stools (Gm) | Source of Inoculum †  | Days from Inoculation to Death | Weight at Autopsy, Gm | Lethargy | Anorexia | Paralysis of Forelegs | Exaltation | Urinary Incontinence | Terminal Hypopyrexia | Paralysis of Hindlegs | Pulmonary Edema, Autopsy | Intestinal Hemorrhage |
|------|--------------------------------------|------------------------|--|-----------------------|--------------------------------|-----------------------|----------|----------|-----------------------|------------|----------------------|----------------------|-----------------------|--------------------------|-----------------------|
| 1    | 10                                   | I O                    | 0.1  | Mouse brain           | 5                              | 102                   |          | +        | +                     | +          |                      |                      |                       | +                        |                       |
| 2    | 10                                   | I O                    | 0.1  | Mouse brain           | 34                             | 104                   |          |          |                       |            |                      |                      |                       |                          |                       |
| 3    | 1                                    | I O                    | 0.06   | Mouse brain           | 106                            | 73                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 4    | 1                                    | I O                    | 0.06   | Mouse brain           | 30                             | 93                    |          |          |                       |            |                      |                      |                       |                          |                       |
| 5†   | 1                                    | I O                    | 0.06   | Mouse brain           | 3                              |                       |          |          |                       |            |                      |                      |                       |                          |                       |
| 6    | 11                                   | Spon                   |  | Contamination         | ?                              | 68                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 7    | ?                                    | Spon                   |  | Contamination         | ?                              | 118                   | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 8    | 1                                    | Spon                   |  | Dirt mole 7           | 2                              | 64                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 9    | 21                                   | Spon                   |  | Dirt moles 7 and 8    | 2                              | 127                   | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 10   | 1                                    | I O                    | 0.1  | C N S mole 2          | 14                             | 81                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 11   | 45                                   | Spon                   |  | Dirt moles 7, 8 and 9 | 23                             | 56                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 12   | 20                                   | I O                    | 0.1  | Human C N S           | 28                             | 97                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 13   | 19                                   | I O                    | 0.1  | Human C N S           | 96                             | 66                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 14   | 1                                    | I O                    | 0.1  | C N S mole 9          | 8                              | 66                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 15   | 1                                    | I O                    | 0.1  | C N S mole 9          | 8                              | 73                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 16   | 39                                   | Spon                   |  | Dirt mole 14          | 27                             | 63                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 17   | 27                                   | Spon                   | 0.1  | Dirt mole 15          | 20                             | 56                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 18   | 31                                   | I O                    | 0.1  | C N S mole 15         | 70                             | 89                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 19   | 30                                   | I O                    | 0.1  | C N S mole 14         | 4                              | 71                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 20   | 8                                    | Spon                   |  | Exposure?             | <10                            | 63                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 21   | 9                                    | I O                    | 0.1  | C N S mole 19         | 47                             | 96                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 22   | 4                                    | I O                    | 0.1  | C N S mole 12         | 39                             | 72                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 23   | 41                                   | I O                    | 0.1  | C N S mole 10         | 80                             | 55                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 24   | 1                                    | S O                    | 1.0  | Human C N S           | 2                              | 92                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 25   | 1                                    | I V                    | 0.3  | Human C N S           | <1                             | 108                   | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 26   | 1                                    | I O                    | 0.1  | Human C N S           | 124                            | 14                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 27   | 1                                    | I O                    | 0.1  | Human C N S           | <1                             | 77                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 28   | 1                                    | I N                    | 0.5  | Human C N S           | 62                             | 79                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 29   | 3                                    | I N                    | 0.5  | Human C N S           | 18                             | 80                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 30   | 64                                   | I V                    | 0.3  | Human C N S           | 139                            | 75                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 31   | 8                                    | I O                    | 0.1  | C N S mole 22         | 2                              | 89                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 32   | 1                                    | I O                    | 0.1  | C N S mole 21         | 54                             | 56                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 33   | 3                                    | I O                    | 0.1  | Mouse brain           | 30                             | 60                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 34   | 4                                    | I O                    | 0.1  | Mouse brain           | 2                              | 62                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 35   | 3                                    | I O                    | 0.1  | Mouse brain           | 2                              | 84                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 36   | 1                                    | I O                    | 0.1  | Mouse brain           | 1                              | 84                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 37   | 65                                   | Exp                    | 20 Gm  | Human stool           | 4                              | 60                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 38   | 56                                   | Exp                    | 20 Gm  | Human stool           | 31                             | 10                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 39   | 3                                    | Exp                    | 8 Gm   | Human stool           | 9                              | 89                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 40§  | 7                                    | I O                    | 0.1  | Human stool           | 27                             | 68                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 41   | 41                                   | I O                    | 0.1  | Human stool           | 22                             | 57                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 42   | 42                                   | I O                    | 0.1  | Human stool           | 5                              | 59                    | +        |          | +                     |            |                      |                      |                       |                          |                       |
| 43§  | 1                                    | I O                    | 0.1  | Human stool           | 5                              | 59                    | +        |          | +                     |            |                      |                      |                       |                          |                       |

\* I O means Intracerebrally, Spon, spontaneously, S O, subcutaneously, I V, intravenously, I N, intranasally, Exp, exposed  
† C N S means central nervous system  
‡ No autopsy was made  
§ The mole was alive still at the end of the experiment

The time of survival is variable and unpredictable, regardless of the route of inoculation or the source of the inoculum or the relative position of the animal in series passage. Animals have died in from 98 hours to 139 days and still demonstrated convincing pictures of one or the other of the two types of pictures seen terminally.

The most reliable results were found in the animals which were inoculated intracerebrally or which were exposed to feces from a human patient who had poliomyelitis or to dirt previously contaminated by an infected animal. For the latter group (moles 6, 7, 8, 9, 10 and possibly 20) the survival time varied from 2 to 23 days of known exposure. All of these animals terminally demonstrated to a convincing degree either paralysis of the extremities or an overwhelming involvement of the so-called vagal type.

The series of animals inoculated by other routes (intranasal, intravenous and subcutaneous) is too small and the survival times and other results too equivocal to warrant conclusions. All such routes, and in addition the intraperitoneal route, should be investigated more thoroughly.

*Miscellaneous Findings or Observations*—For a mole to come to the surface is abnormal behavior, as they are wary animals and come to the surface only to drink when the laboratory is quiet. Of the 41 moles to die to date in this experiment, only 6 have died beneath the surface of the dirt.

Anorexia, as judged by the leaving of meat on the surface, was noted in 26 moles. Lethargy was noted in 23 and, of course, could not be observed in animals undergoing a fulminating type of death during the night. Paralysis of extremities already has been discussed. Excitability was manifested in several ways, many moles came to the surface and ran around for hours—mole 17 ran backward, others showed hyperactivity on casual stimulation, the constant running motion of the hindlegs, with the mole on its back, was obviously the result of cortical stimulation.

Fourteen moles remained for a considerable time on top of the dirt, others found dead on the surface in the morning undoubtedly were showing this abnormal behavior for some time before death. Hyperpyrexia was detected only nine times but undoubtedly would have been found more frequently had temperatures been taken more often. Unfortunately, moles are difficult to keep under laboratory conditions, and it seemed advisable to minimize their handling. Rectal temperatures of 37.5 to 38.5 C (99.5 to 101.3 F) were regarded as hyperpyrexia. This elevation of temperature frequently antedated any signs of lethargy or paralysis. Terminal hypopyrexia was encountered 10 times, and probably all animals would exhibit this feature terminally were more frequent temperatures taken. Rectal temperatures below 34.5 C (94.1 F) were considered as hypopyrexia.

Cardiac arrhythmia was observed in the course of taking temperatures of moles 9 and 42. In the former it consisted of the dropping of every fourth beat, in the latter it consisted of frequent extrasystoles. This feature should be given more thorough investigation, especially in animals showing a terminal picture suggesting overwhelming vagal involvement.

The remaining symptoms enumerated in table 1 are self explanatory. Fleas, and occasionally mites were observed in the fur of 9 animals. The percentage of moles so infested is undoubtedly much higher than this would indicate, as the parasites readily leave their host when the body becomes cold and, consequently, were not found on moles dying during the night or those placed in the ice box prior to autopsy. Their presence is noted here because they may conceivably play a role in the dissemination of disease herein referred to as probably due to exposure to contaminated dirt.

The enumeration of cases in which pulmonary edema and intestinal hemorrhage occurred is based solely on gross observations. The incidence of these pathologic findings would probably be increased by microscopic examination.

*Pathologic Observations*—Neuronophagia and inflammatory cell reaction in the meninges or perivascular spaces were entirely lacking in all moles and in the Swiss mice and cotton rats in which passage was effected. Practically all animals showed some degree of vascular engorgement. In most animals there were profound degenerative changes of the cells of the anterior horns of the spinal cord and, to a lesser degree, of the nerve cells elsewhere in the spinal cord, the brain stem, the cerebellar nuclei and the cerebral cortex. The earliest phase of this change is a dissolution of the Nissl substance, followed by a pale, powder blue staining of the cytoplasm, an indistinctness of the cytoplasmic membrane, vesicularity and chromatolysis of the nucleus and eventually an almost complete ablation of the entire nerve cell. By correlating the degree of changes with the maximum possible postmortem time before fixation and carefully studying control material left at room and ice box temperatures for known periods before fixation one became convinced that the changes were autolytic rather than cytolytic.

TABLE 2—*Observations on Control Moles*

| Control mole  | 1 | 2 | 3 | 4  | 5  | 6  | 7  | 8 | 9  | 10 | 11 | 12  | 13 | 14 | 15 |
|---|---|---|---|----|----|----|----|---|----|----|----|-----|----|----|----|
| Days in captivity before being used                   | 0 | 0 | 1 | 3  | 2  | 0  | 0  | 1 | 25 | 0  | 0  | 100 | 47 | 4  | 4  |
| Hours in ice box post mortem before fixation          | 0 | 0 | 0 | 23 | 24 | 0  | 0  | 0 | 0  | 4  | 4  | 15  | 3  | 0  | 4  |
| Hours at room temperature post mortem before fixation | 0 | 0 | 0 | 0  | 0  | 24 | 16 | 8 | 4  | 5  | 5  | 0   | 0  | 4  | 18 |
| Inflammatory meningeal reaction                       | 0 | 0 | 0 | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | +   | +  | 0  | 0  |
| Degree of autolysis of anterior horn cells *          | 1 | 1 | 0 | 2  | 3  | 4  | 4  | 4 | 3  | 3  | 3  | 1   | 1  | 2  | 4  |

\* 0 means no autolysis, 1, dissolution of Nissl substance and indefiniteness of the cytoplasmic membrane in a few cells, 2, chromatolysis and lysis of Nissl substance affecting one third to one half of the cells, 3, same as 2, except that one half to two thirds of the cells were involved, 4, autolytic changes in all cells of the anterior horns.

In 6 moles of the experimental series (6, 15, 16, 22, 23 and 25) petechial hemorrhages were noted at various sites throughout the central nervous tissue.

*Control Observations*—Fifteen moles were used as various types of controls as indicated in table 2. Of these controls, moles 1 to 3 were examined immediately after their deaths, and others were left at room or ice box temperatures for varying periods, to ascertain whether the changes found in the anterior horn cells of inoculated or exposed moles were the result of cytolysis or autolysis. A careful comparison of the changes found in the control and the experimental series leaves no doubt that they are autolytic in character.

The lack of inflammatory cell reaction in the central nervous tissues of the experimental animals and the impression of a paucity of leukocytes in the vascular channels raised the question whether the mole was capable of mobilizing leukocytes in response to an inflammatory stimulus. Control moles 12 and 13 were inoculated intracerebrally with a saline suspension of fresh blood agar subculture of *Pneumococcus* type I. Microscopic sections of this material revealed a definite ability to respond with polymorphonuclear leukocytes.

Control moles 14 and 15 were studied to rule out several theoretic possibilities. In all respects these 2 animals were fed and handled as were the animals of the experimental series except that they were isolated two floors distant from the

other experimental animals. First, it seemed advisable to check on a possible avitaminosis resulting from the artificial diet of captivity, second, it was conceivable that the trauma of daily handling and temperature taking might lead to a bizarre heretofore undescribed illness, and third, it seemed advisable to rule out the possibility of the animals reacting to the inoculation of foreign proteins. These 2 animals were inoculated both intracerebrally and intravenously with brain and spinal cord from 2 moles which were killed almost instantly by a fracture of the spinal column when caught. Control mole 15 died after 36 days, and autopsy revealed extensive hemorrhagic pneumonia and peritonitis. Control mole 14, expired 101 days after inoculation, autopsy revealed acute trichiniasis. Therefore, it does not seem likely that death and the symptoms so consistently found in the experimental group could be due to dietary deficiency, to trauma of daily handling or to a foreign protein reaction. Furthermore, many animals have been kept as stock moles for periods of 30 to 100 days with no evidence of dietary deficiency.

*Passage from Moles to Swiss Mice and Cotton Rats*—Each intracerebral passage was made with a 1:10 saline dilution of a filtrate of wet mole brain and spinal cord. The filtrates were obtained by light centrifugation of the material and passage through a Chamberland L-5 filter. Subsequent mouse and cotton rat passages were made with diluted filtrates of material similarly treated. Mice received 0.03 cc and cotton rats 0.05 cc of diluted filtrate. In all 39 Swiss mice and 35 cotton rats were so inoculated, either directly with mole tissue filtrate or with subsequent mouse or cotton rat tissue filtrate. Of these, 27 Swiss mice and 17 cotton rats still survived after periods varying from 15 to 69 days. Moles 2, 3, 9 and 21 were used as sources of the inoculums of four series. Infectivity has been demonstrated through three serial passages from moles 2 and 9, and through but one passage from moles 3 and 21. However, the original passage of material from mole 9 resulted in 1 cotton rat dying in 17 days, from which subsequent passage was successful, while 4 mice and 2 more cotton rats succumbed within 5 days of each other 60 to 65 days after injection.

As in the experimental mole series, no neuronophagia, perivascular lymphocytic cuffing or meningeal inflammatory cell reaction was found on microscopic examination of tissues of the central nervous system.

*Failure to Effect Passage to Monkeys*—Intracerebral inoculation of 0.25 cc of a 1:10 saline dilution of the filtered wet brain and spinal cord of mole 19 failed to produce paralysis or untoward symptoms in an old rhesus monkey during the course of 120 days of observation. As nothing was known of the past history of the monkey other than that she had been used for the production of anti-Rh serum, it was decided to use recently imported young rhesus monkeys and larger doses, comparable to the doses used by Marks.<sup>8</sup> Accordingly, on July 28, 1948, 2.0 cc of a 1:10 saline dilution of the filtered wet brain and spinal cord of mole 38 was injected intracerebrally into a young rhesus monkey. (The filtrate had been obtained by passing the material through a Berkefeld N candle.) On July 30 the same amount of inoculum, similarly prepared, from mole 42 was injected intracerebrally into another recently imported young rhesus monkey. Neither of these animals has shown paralysis or any untoward symptoms after 220 to 222 days of observation.

#### COMMENT

This report is destined to be controversial, as lesions typical of poliomyelitis were lacking. The lack is by no means unique in the mole,

<sup>8</sup> Marks, H. K. J. Exper. Med. **14** 116, 1911.

similar results having been reported by various investigators attempting to transmit poliomyelitis to other animals

Marks<sup>8</sup> reported convulsions and death observed in rabbits after inoculation of poliomyelitis virus, yet found no lesions comparable to those observed in man and monkey in the tissues of the central nervous system. He did, however, succeed in passing the virus from 3 rabbits to 3 monkeys by massive intracerebral inoculation (40 cc, 30 cc and 25 cc) and demonstrated classic lesions in nerve tissue. Marks's rabbits underwent no paralysis but succumbed after convulsive seizures.

Rosenau and Havens<sup>7</sup> reported that 22 of 54 rabbits died, after being inoculated with poliomyelitis virus. Death was preceded by one or the other of two terminal syndromes similar to those exhibited by moles in this series. They also were unable to fix their virus on passage, and the lesions of the central nervous system were quite dissimilar to those observed in man and monkey. The degeneration of large motor cells reported by them may be essentially autolytic in character, as no controls were employed for comparison. They succeeded in passing the disease-producing agent to a monkey, which died of a respiratory rather than a typical paralytic syndrome. The pathologic condition observed in the monkey resembled that in the rabbit and was far short of a true duplication of the usual human or simian lesions. A quotation from their article is pertinent at this point:

If the virus of poliomyelitis may be so altered in the rabbit as scarcely to be recognizable, may it not be still more profoundly changed in other animals? The conjecture then arises that poliomyelitis, instead of being limited naturally to man and experimentally to monkeys, may in fact occur in other animals in unnoticed or unrecognized form."

Sandler<sup>6</sup> also adapted the rabbit to poliomyelitis virus by first inducing hypoglycemia by starvation and/or insulin. Pathologically he found no neuronophagia or perivascular or meningeal cellular infiltration. He described intracellular changes which might represent autolysis. He was able to produce typical lesions in the monkey by inoculating the infected rabbit material. Paralysis was evident in only 2 of 11 animals.

Hassin<sup>9</sup> concluded that the chief pathologic changes found in the rabbit are of a cellular degenerative rather than of a cellular infiltrative or neuronophagic type. In paralysis produced in sheep and believed by Hassin to be poliomyelitic, the pathologic changes were not uniform but tended to simulate more closely those found in man and monkey than those produced in rabbits. Hassin's results must be interpreted with caution, however, as it is not stated whether the inoculums were filtered material, no attempt seems to have been made to differentiate between autolytic and cytolytic cellular changes, and

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9 Hassin, G. B. *M. Rec.* 92:89, 1917

in 2 sheep and 1 rabbit cerebrospinal fluid appears to have served as the infective inoculum. It is now rather generally accepted that the virus cannot be recovered from this source. He concluded, however, that the replica of the histopathologic changes observed in man and in the monkey is not found in the rabbit or the sheep.

A slight variation from the classic histopathologic picture of poliomyelitis has been reported observed in the guinea pig. In 1913 Neustaedter<sup>10</sup> reported that 2 guinea pigs became spontaneously paralyzed after rather undeniable exposure to a monkey ill of experimental poliomyelitis. On first passage of spinal cord from one of the guinea pigs to a second guinea pig no perivascular infiltration was found and only slight neuronophagocytosis, but on subsequent passage through other guinea pigs these features reappeared in classic form.

Dalldorf and Sickles<sup>11</sup> recently reported the isolation of a virus from the stools of 2 patients who had poliomyelitis, which produced paralysis in suckling mice and hamsters with an absence of lesions of the central nervous system. They described severe and widespread changes occurring in skeletal muscles. Changes described as a loss of striations, acidophilic reaction and fragmentation could be autolytic in character. However, they described intense proliferation of young muscle cells with endothelial cell phagocytosis. Their report prompted a search for similar changes in the moles herein concerned. Muscles from the abductor group of the forelegs and the diaphragm were removed from the carcasses, after having been fixed in formaldehyde solution. No such changes could be identified with certainty in any of the animals of the experimental or the control series.

Armstrong<sup>12</sup> showed, and others have confirmed, that the cotton rat is susceptible to the Lansing strain of poliomyelitis virus, which produces in this rat microscopic lesions in every respect similar to those observed in man and monkey. Toomey, Takacs and Weaver<sup>13</sup> encountered an exception to this finding when they were identifying what appears to have been a strain of poliomyelitis virus isolated from a creek in Perryville, Ohio, in 1944. On several occasions their animals died without microscopic lesions that could be identified as those of poliomyelitis, yet they were successful in producing the classic lesions in 2 monkeys and in many of their cotton rats.

In the past critics have been reluctant to accept as poliomyelitis in animals a paralytic syndrome that fails to be accompanied by the classic lesions. It may well be that they have been too rigid in their expect-

10 Neustaedter, M. J. A. M. A. 60 982, 1913

11 Dalldorf, G. and Sickles, G. Science 108 61 1948

12 Armstrong, C. Pub. Health Rep. 54 1719, 1939



tations in this respect and consequently may have overlooked the natural existence of the disease in other species, as Rosenau and Havens cautioned us 32 years ago

A second controversial point raised by the work herein reported is the possibility that an epizootic virus disease of either the mole or the mouse has been brought to the fore. The Bureau of Animal Industry of the United States Department of Agriculture<sup>13</sup> reports that no epizootic disease of an encephalitic type is known to occur among moles. Furthermore, the inoculum used on all occasions in this experiment was from a known poliomyelitic source. That an epizootic disease of mice might have been present in the mouse brain used is theoretically possible. However, typical lesions of poliomyelitis have been produced repeatedly by injecting this strain into Swiss mice. Were one to raise the question of the possible presence of Theiler's mouse virus one would have to admit, and explain, the reversal of the virus' tendency to produce constantly paralysis of the hindlegs to a tendency to involve the forelegs more commonly.

In addition to the four a priori concepts suggesting that the mole might be a natural reservoir of poliomyelitis, two facts have been brought out by the experiments performed to date that lend further support to such a hypothesis. First, cross infection between moles in casual contamination is apparent, and, second, the frequently encountered long survival times of some animals would greatly facilitate the subterranean harboring of the disease in epizootic form between epidemics among human beings. Were the disease to be found occurring among moles in conjunction with an epidemic among human beings, one would have a fair explanation as to why the incidence among males is somewhat greater than that among females, "for little boys are much more likely to be playing in the dirt than are little girls."

It is realized that there are many interesting and important phases of this problem that have not been investigated, and it is with sincere apologies that the work is reported with so much still to be done. It would seem to be important to learn (1) to which strains of the virus the mole is susceptible, (2) by what routes of inoculation infection is possible, (3) whether the mole flea (*Paloepsylla gracilis*) is a vector or possible intermediary host, (4) whether typical microscopic lesions of poliomyelitis might not be produced eventually on further serial passage in moles, Swiss mice or cotton rats, and (5) if, and for what periods, the virus might be excreted from the pharynx and in the stools. However, it seems justifiable to publish the data accumulated to date, controversial as they may be, in order that physicians may

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13 United States Department of Agriculture, Bureau of Animal Industry  
Personal communication to the author

be alerted to the advisability of keeping this problem in mind in taking individual case histories, as well as to the advisability of making epidemiologic surveys of moles in conjunction with future epidemics of poliomyelitis

The question is immediately raised whether the mole is susceptible to other virus diseases having a hot weather seasonal incidence. One thinks of rabies, eastern and western equine encephalomyelitis, and epidemic encephalitis of the St. Louis and Japanese types.

#### SUMMARY

Several reasons why the mole might be the natural reservoir of poliomyelitis are enumerated and discussed.

Forty-three moles have been exposed to, or inoculated with, virus of poliomyelitis obtained from the following sources: mouse brain experimentally infected with the rodent-adapted strain of Lansing virus, brain and spinal cord of a human patient who died of poliomyelitis, and stools of four infected human beings. Of these animals, but 2 have survived. Two distinctly different types of terminal behavior are described. The survival time of the infected animals is unpredictable, regardless of the source of the inoculum, the route of inoculation or the relative position of the animal in serial passage. There is no tendency of the virus to become fixed. Possible routes of inoculation other than intracerebral have not been adequately investigated.

Miscellaneous autopsy observations and terminal behavior traits are tabulated and discussed.

Successful passage of the virus from mole to mole and from mole to Swiss mouse and cotton rat is reported. Unsuccessful attempts to pass the virus to 3 monkeys is reported.

The absence of typical histopathologic lesions of poliomyelitis in the moles and the Swiss mice and cotton rats in which passage of mole brain and spinal cord was effective is discussed, and the literature concerning similar experiences of other investigators is reviewed.

Fifteen animals were employed as controls for comparative studies of autolytic changes, dietary deficiency, ability of the mole's brain to respond with mobilization of inflammatory cells, trauma of handling and reaction to foreign protein.

# THE BLOOD CELLS AND THE HEMOPOIETIC AND OTHER ORGANS OF DOGS GIVEN INTRAVENOUS INJECTIONS OF 2-CHLOROETHYL VESICANTS

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**I**N A recent paper the quantitative changes occurring in the leukocytes of the peripheral blood and the histologic changes in the hemopoietic organs of rats poisoned with 2-chloroethyl vesicants were analyzed<sup>1</sup> In the present investigation, data of a similar nature on dogs poisoned with these vesicants are presented Better information concerning the daily changes in the blood picture has been obtained from the dogs, but because of more extensive pathologic changes, the lymph nodes and the spleen did not lend themselves to the same kind of quantitative study as was possible with those of the rats However, it is felt that the data obtained from the poisoned dogs will supplement the information obtained from the rats and advance knowledge concerning the relations between the leukocytes of the peripheral blood and the hemopoietic organs In addition, changes in the daily counts of the red blood corpuscles and the thrombocytes of the peripheral blood, not obtained in the rat, are included in the present paper The data will also, supplement those which have been discussed in relation to the matter of using the vesicants in tumor therapy as presented in the reports given at a symposium of the American Association for the Advancement of Science<sup>2</sup>

## MATERIAL AND METHODS

The material consisted of 17 male dogs which were being used by the biochemistry department of the University of Virginia under the direction of Dr Alfred Chanutin for the study of the effects exerted by the 2-chloroethyl vesicants on the proteins and other constituents of the blood<sup>3</sup> The dogs were fed a stock

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1 Kindred, J E Arch Path **43** 253, 1947

2 Moulton, F R Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster Pa, Science Press Printing Company, 1947, p 95

3 (a) Gjessing, E C, and Chanutin, A J Biol Chem **165** 413, 1946

(b) Chanutin, A, and Ludewig, S ibid **167** 313, 1947

laboratory diet and were kept under observation for at least a month. The animals were in good condition at the beginning of the experimental period. The vesicants used were redistilled sulfur mustard, or bis(2-chloroethyl) sulfide, dissolved in thiodiglycol (code name in this report, H), and the hydrochlorides of ethyl-bis(2-chloroethyl) amine (code name, HN-1) and tris(2-chloroethyl) amine (code name, HN-3), dissolved in isotonic sodium chloride solution just before being injected into the saphenous vein. Blood was drawn from the jugular vein for blood counts and smears. At least 2 dogs were started with each vesicant, and if they did not die as a result of the single injection, they were given further injections of the same or increased amounts of the vesicant, or the experiment was discontinued. As a result of the further injections there were fourteen different conditions of dosage to which these dogs were subjected. The amounts and the times of dosage are given in the tables. The vesicants were injected in about 0.4 cc of solvent.

TABLE 1—Blood Effects of Intravenously Injected Bis(2-Chloroethyl) Sulfide (Code Name, H)\*

| A White Blood Corpuscles                |                          |           |                             |                        |                     |               |                             |                           |                  |  |
|---|--------------------------|-----------|-----------------------------|------------------------|---------------------|---------------|-----------------------------|---------------------------|------------------|--|
| Day                                     | White Blood Corpuscles † |           | Neutrophilic Granulocytes † |                        |                     | Lymphocytes † | Eosinophilic Granulocytes † | Basophilic Granulocytes † | Metarubricytes ‡ |  |
|   | Counts                   | Mean      | Counts                      | Mean                   | Percentage Immature |               |                             |                           |                  |  |
| Prior to injection (control)            | 6                        | 100 ± 2.4 | 6                           | 100 ± 2.6              | 15.0 ± 2.5          | 100 ± 7.1     | 100 ± 6.4                   | 0.600                     | 0.600            |  |
| After injection                         |                          |           |                             |                        |                     |               |                             |                           |                  |  |
| 1                                       | 2                        | 65 ± 4.0§ | 6                           | 63 ± 1.9§              | 13.0 ± 2.4          | 54 ± 6.0§     | 100 ± 35.0                  | 16.000                    | 0.600            |  |
| 2                                       | 2                        | 63 ± 6.0§ | 6                           | 69 ± 4.1§              | 8.0 ± 1.2§          | 20 ± 5.4§     | 117 ± 23.0                  | 5.600                     | 0.600            |  |
| 3                                       | 2                        | 54 ± 7.0§ | 6                           | 62 ± 5.7§              | 11.0 ± 1.1          | 26 ± 8.0§     | 40 ± 6.4§                   | 7.600                     | 0.600            |  |
| 4                                       | 2                        | 47 ± 0.0§ | 6                           | 52 ± 4.0§              | 9.5 ± 0.8§          | 29 ± 10.0§    | 34 ± 19.0§                  | 2.600                     | 0.600            |  |
| 5                                       | 2                        | 45 ± 2.0§ | 6                           | 46 ± 1.9§              | 8.8 ± 2.2§          | 30 ± 9.0§     | 35 ± 9.2§                   | 1.600                     | 0.600            |  |
| 7                                       | 2                        | 23 ± 7.0§ | 6                           | 17 ± 2.8§              | 21.0 ± 2.5          | 30 ± 7.0§     | 27 ± 11.0§                  | 0.600                     | 14.600           |  |
| B Red Blood Corpuscles and Thrombocytes |                          |           |                             |                        |                     |               |                             |                           |                  |  |
|   |                          | Day       | Counts                      | Red Blood Corpuscles † | Thrombocytes †      |               |                             |                           |                  |  |
| Prior to injection (control)            |                          |           | 6                           | 100 ± 0.7              | 100 ± 1.1           |               |                             |                           |                  |  |
| After injection                         |                          | 15        | 10                          | 94 ± 2.3§              | 97 ± 4.1            |               |                             |                           |                  |  |
|   |                          | 7         | 2                           | 81 ± 4.0§              | 52 ± 15.0§          |               |                             |                           |                  |  |

\* Dogs 11 and 12 were used. The dose was 0.3 mg per kilogram of body weight.

† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standard error.

‡ The incidence per number of white blood cells counted in smears is given.

§ The number is significantly less than the control mean.

Blood counts and smears were made daily when possible during the first week after injection and on several days of the following weeks. On the death of the animal, the mesenteric and peripheral lymph nodes, the spleen, the adrenal glands and the thymus were removed in all cases, in most cases the tonsils were taken, and in some cases pieces of liver, ileum, cecum and colon, and in 1 case pieces of pancreas and kidney, were taken. The spleen and the adrenal glands were weighed. These organs were fixed in Helly's fluid, and sample sections were stained with hematoxylin and eosin, Feulgen's stain or Mallory's stain. The sternum and the ribs were split, and touch smears of the marrow were made and stained with the May-Giesma method, other parts of the bone and marrow were fixed in Helly's fluid, decalcified, sectioned and stained as were the other organs. The organs of 4 young dogs were used as controls. The methods by which quantitative studies were made of the organs are given in their respective sections and in the tables. The standard errors of the means of the values

TABLE 2—Blood Effects of Intravenously Injected Ethyl-Bis(2-Chloroethyl) Amine (Code Name, HN-1)\*

| A White Blood Corpuscles     |                          |        |                             |        |                     |               |                             |                           |                   |
|------------------------------|--------------------------|--------|-----------------------------|--------|---------------------|---------------|-----------------------------|---------------------------|-------------------|
|                              | White Blood Corpuscles † |        | Neutrophilic Granulocytes † |        | Percentage Immature | Lymphocytes † | Eosinophilic Granulocytes † | Basophilic Granulocytes † | Meta rubricytes ‡ |
|                              | Day                      | Counts | Mean                        | Counts |                     |               |                             |                           |                   |
| Prior to injection (control) | 9                        |        | 100 ± 15.0                  | 9      | 100 ± 18.0          | 22.0 ± 2.3    | 100.0 ± 15.0                | 100.0 ± 31.0              | 0.900             |
| After injection              | 2                        | 2      | 58 ± 17.0§                  | 6      | 64 ± 9.2§           | 9.5 ± 2.3§    | 4.7 ± 1.7§                  | 35.0 ± 14.0§              | 0.600             |
|                              | 3                        | 2      | 47 ± 9.0§                   | 6      | 53 ± 5.0§           | 7.9 ± 0.9§    | 14.0 ± 6.1§                 | 60.0 ± 19.0               | 0.600             |
|                              | 4                        | 2      | 40 ± 15.0§                  | 6      | 47 ± 6.0§           | 20.0 ± 3.5    | 6.7 ± 2.7§                  | 6.0 ± 3.7§                | 0.600             |
|                              | 5                        | 2      | 18 ± 10.0§                  | 6      | 20 ± 6.0§           | 35.0 ± 9.5    | 9.0 ± 1.2§                  | 4.0 ± 2.4§                | 0.600             |
|                              | 6                        | 2      | 16 ± 0.0§                   | 6      | 13 ± 0.8§           | 34.0 ± 5.7    | 35.0 ± 3.1§                 | 3.2 ± 0.9§                | 0.600             |
|                              | 11                       | 2      | 61 ± 6.0§                   | 6      | 64 ± 3.0§           | 25.0 ± 2.2    | 40.0 ± 12.0§                | 46.0 ± 12.0§              | 0.600             |
|                              | 25                       | 31     | 43 ± 8.0§                   | 6      | 46 ± 3.0§           | 17.0 ± 2.4    | 30.0 ± 6.7§                 | 31.0 ± 13.0§              | 0.600             |
|                              | 39                       | 46     | 95 ± 5.0                    | 6      | 100 ± 2.5           | 24.0 ± 1.7    | 70.0 ± 8.3                  | 61.0 ± 27.0               | 0.600             |

## B Red Blood Corpuscles and Thrombocytes

|                              | Day | Counts | Red Blood Corpuscles † | Thrombocytes † |
|------------------------------|-----|--------|------------------------|----------------|
| Prior to injection (control) |     | 10     | 100 ± 2.6              | 100 ± 4.5      |
| After injection              | 2   | 2      | 82 ± 0.0*              | 112 ± 5.0      |
|                              | 3   | 4      | 80 ± 2.0*              | 55 ± 12.0*     |
|                              | 5   | 4      | 75 ± 2.6*              | 19 ± 3.0*      |
|                              | 11  | 46     | 87 ± 4.0*              | 90 ± 8.9       |

\* Dogs 5 and 6 were used (dog 6 died on the sixth day). The dose was 1.0 mg per kilogram of body weight.

† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standard error.

‡ The incidence per number of white blood cells counted in smears is given.

§ The number is significantly less than the control mean.

TABLE 3—Blood Effects of Intravenously Injected Tris(2-Chloroethyl) Amine (Code Name, HN-3)\*

| A White Blood Corpuscles     |                          |        |                             |        |                     |               |                             |                           |                   |
|------------------------------|--------------------------|--------|-----------------------------|--------|---------------------|---------------|-----------------------------|---------------------------|-------------------|
|                              | White Blood Corpuscles † |        | Neutrophilic Granulocytes † |        | Percentage Immature | Lymphocytes † | Eosinophilic Granulocytes † | Basophilic Granulocytes † | Meta rubricytes ‡ |
|                              | Day                      | Counts | Mean                        | Counts |                     |               |                             |                           |                   |
| Prior to injection (control) | 16                       |        | 100 ± 2.1                   | 16     | 100 ± 2.7           | 18 ± 3.1      | 100 ± 5.0                   | 100 ± 6.6                 | 0.1600            |
| After injection              | 1                        | 3      | 59 ± 3.6§                   | 36     | 89 ± 4.7            | 14 ± 1.4      | 10 ± 0.9§                   | 45 ± 6.8§                 | 2.3600            |
|                              | 4                        | 7      | 15 ± 1.8§                   | 36     | 19 ± 1.6§           | 31 ± 3.7#     | 9 ± 0.8§                    | 14 ± 1.7§                 | 1.3600            |
|                              | 8                        | 9      | 38 ± 11.0§                  | 8      | 25 ± 13.0§          | 60 ± 7.8#     | 16 ± 4.0§                   | 15 ± 3.6§                 | 0.800             |
|                              | 10                       | 14     | 60 ± 5.0§                   | 16     | 94 ± 6.8            | 36 ± 2.7#     | 21 ± 4.1§                   | 35 ± 7.0§                 | 0.1600            |
|                              | 15                       | 45     | 73 ± 5.2§                   | 26     | 100 ± 6.3           | 26 ± 1.8      | 27 ± 3.3§                   | 72 ± 8.4                  | 4.2600            |

## B Red Blood Corpuscles and Thrombocytes

|                              | Day | Counts | Red Blood Corpuscles † | Thrombocytes † |
|------------------------------|-----|--------|------------------------|----------------|
| Prior to injection (control) |     | 16     | 100 ± 0.7              | 100 ± 2.7      |
| After injection              | 1   | 3      | 100 ± 0.9              | 106 ± 2.7      |
|                              | 4   | 5      | 92 ± 2.0*              | 96 ± 3.1       |
|                              | 7   | 14     | 81 ± 2.1*              | 55 ± 5.4*      |
|                              | 15  | 45     | 80 ± 2.3*              | 91 ± 5.0       |

\* Dogs 1 and 2 were used through the forty-fifth day, dogs 13 and 14, through the fourteenth day. The dose was 1.0 mg per kilogram of body weight.

† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standard error.

‡ The incidence per number of white blood cells counted in smears is given.

§ The number is significantly less than the control mean.

# The number is significantly greater than the mean of the control.

measured were calculated wherever possible and appear in the tables following the means. At the suggestion of the editor an attempt has been made to make the nomenclature of the blood cells conform with the revisions made by the Committee for Clarification of Cells and Diseases of the Blood and Blood-Forming Organs<sup>3b</sup>

#### EXPERIMENTAL RESULTS

*White Blood Corpuscles of the Peripheral Blood*—The relative changes in the distributions of the total number of white blood corpuscles and of the neutrophilic granulocytes, lymphocytes and eosinophilic granulocytes per cubic millimeter of peripheral blood in the dogs poisoned with the 2-chloroethyl vesicants are presented in tables 1 to 3. The averaged counts are listed as percentages of the means of counts made several days before injection (control means). Each count of each dog was calculated in percentage of the average control count of that dog. The percentages were then averaged with the percentages of the other dogs in the group and the results listed as the mean percentages with standard errors as given in the tables. When only one count was available, the standard error entered was that for the control count. The total counts of the white blood cells were made with standard blood-counting apparatus. The total counts of granulocytes and lymphocytes were calculated from the percentage distributions of these cells in the smears and from the total white blood cell counts. At least three differential counts of 100 cells per count were made for each mean listed in the tables. The immature neutrophils are accounted for as mean percentages of their incidences in the neutrophilic populations of the smears. The method of presenting the data in percentages of the respective means of the controls was used for the purpose of contrasting the relative changes between the several groups of poisoned dogs. Because of the low incidence of monocytes and of basophilic granulocytes in the normal blood, no records of the distributions of the monocytes are entered in the tables, but the incidences of the basophils have been entered to show trends, although their distributions are not statistically significant.

In the following discussion the data will be presented with the idea of showing, if possible, the respective roles of the several types of cells in the total picture. Also, for brevity, the qualification of milligrams per kilogram of body weight will be understood to follow the numerical reference to the amount of vesicant intravenously injected. From analysis of the data presented in tables 1 to 3 the differential effects of the vesicants on the counts of the white blood corpuscles of dogs which survived for at least a week after the initial injection have been contrasted. H (03) (table 1) caused leukopenia immediately as a result of moderate neutropenia and lymphopenia. The neutropenia gradually became more severe and was greater at the end of the week than at the beginning, while the lymphopenia did not change. Eosinophilic granulocytes decreased slowly, and there was no marked shift to the left of the immature neutrophils until the seventh day. Marked basophilia occurred during the first three days. Re-injection of H (03) on the seventh day after the initial injections aggravated these conditions and was fatal.

HN-1 (10) caused leukopenia immediately as a result of moderate neutropenia and extremely severe lymphopenia (table 2). The neutropenia gradually increased in severity, and there was a marked shift to the left of the immature neutrophils on the fourth day, which continued through the seventh day. There was alleviation of the lymphopenia on the seventh day. Eosinophils decreased more rapidly and reached a lower level than in the H (03) poisoned dogs. There was no basophilia.

The effects of HN-3 (10) (table 3) on the white blood cells are differentiated from those in both the H (03) and HN-1 (10) poisoned dogs by the moderate initial leukopenia produced only by severe lymphopenia, by the severe acute neutropenia on the fourth day, and by the marked shift to the left of the immature neutrophils on the fourth day. The effects of HN-3 (10) agree with those of HN-1 (10) and not with those of H (03) in producing a rapid decrease in eosinophils and in not affecting the basophils.

From these results it would appear that H (03) and HN-1 (10) have a moderate immediate inhibitory effect on the bone marrow's production of neutrophils. HN-3 (10) has a slower but more harmful effect. HN-1 (10) and HN-3 (10) have a greater inhibitory effect on the production of lymphocytes than does H (03).

In dogs poisoned with H (05) the initial neutropenia was relatively greater than after H (03), but the lymphopenia was of about the same degree. The eosinophils were also more depressed by the increase in the dose. Basophilia was not so marked as after H (03). In the dogs poisoned with H (10) there were initially severe lymphopenia and significant decreases in eosinophils and immature neutrophils. Basophilia was present. Significant neutropenia did not occur until the third day and was acute. All the cells decreased on this day. On the fourth day there were practically no leukocytes in the blood. These conditions indicate that increase in the amount of H injected has a greater initial effect on the lymphocytes than on the granulocytes, but neutropenia occurs acutely and is much more severe than that caused by smaller amounts of the vesicant. The terminal conditions are more drastic than those following repetition of H (03).

After the first week, beyond which only the HN-1 (10) and HN-3 (10) poisoned dogs were followed without further injections, the remission of the neutropenia began sooner in the HN-3 (10) dogs than in the dog poisoned with HN-1 (10), and the neutrophils reached control level by the tenth day in contrast with the thirty-ninth day in the HN-1 (10) poisoned dog. On the other hand, a slight remission of the lymphopenia occurred in the HN-3 (10) dogs, compared with the practical alleviation of the lymphopenia in the HN-1 (10) dog. In both groups the eosinophils fluctuated throughout the remainder of the period, and although they increased, their distributions were much more variable than those of the other cells. The shift to the left of the immature neutrophils continued for a longer period in the HN-3 (10) dogs than in the HN-1 (10) dog.

No further experiments were carried on with HN-1, but dogs given an initial injection of HN-3 (10) were given further injections of the same amount or increasing amounts of HN-3. No detailed description of the conditions incident to these injections will be given here, but so long as the dogs survived the further injections all had the same pattern of severe initial lymphopenia, and most showed delayed but severe neutropenia on the third and fourth days, followed by more rapid remission of the neutropenia than of the lymphopenia. Also, a shift to the left of the immature neutrophils occurred before remission of the neutropenia. In 1 dog which was repeatedly given HN-3, there was continuous basophilia through the course of several injections, but it gradually faded out. Increasing the amount of HN-3 to 12 and 15 mg per kilogram and giving it in single injections produced lethal effects, but the initial changes in the white blood cells were the same as those following smaller doses. Quantitative study of the smears showed that there was no evidence of increase of degenerated white blood cells in the blood of the poisoned dogs. There were no changes in the incidences of the monocytes of the peripheral blood during the experimental period.

*Red Blood Corpuscles of the Circulating Blood*—The red blood corpuscles of the circulating blood were counted by standard methods, and the counts are presented as percentages of control counts as were those of the white blood corpuscles. Part B of tables 1 to 3 shows that mild anemia followed poisoning of the dogs HN-1 (10) immediately caused moderate anemia, H (03), mild anemia, but HN-3 (10) had no immediate effect. Subsequently, the anemia in the HN-1 (10) poisoned dogs remained at the same level, but in the H and HN-3 poisoned dogs the red blood corpuscles decreased in number until the degree of anemia was equal to that in the HN-1 poisoned dogs. During the periods when the red blood corpuscles of these dogs were studied, there was no remission of the anemia, and it became slightly more severe if the dogs were given further injections of the same or larger amounts of HN-3. The conclusion is drawn that a certain number of erythropoietic cells are injured initially, and although the bone marrow may regenerate, there is never sufficient recovery of the production to replace the lost cells. The crises in marrow regeneration are indicated by the showers of metarubricytes in the peripheral blood which occurred on the seventh day in the H (03) poisoned dogs (table 1, A), from the ninth to the fifteenth day in the HN-3 (10) group (table 2, A) and not until the sixth week in the HN-1 (10) poisoned dog (table 3, B). Quantitative study of the sizes of the red blood corpuscles in the smears showed that there were no differences between the incidences of variations in size of these before and after poisoning.

*Thrombocytes of the Circulating Blood*—The thrombocytes were counted in the usual manner and counts were made at the same time that the red blood corpuscles were counted. All the vesicants produced thrombopenia. The distributions of the thrombocytes are listed in part B of tables 1 to 3. In contrasting the effects of the several agents it will be seen that HN-1 (10) (table 2, B) produced thrombopenia sooner after injection than did H (03) (table 1, B) or HN-3 (10) (table 3, B). About the same percentage decrease occurred in all groups, but in those poisoned with HN-1 (10) remission was more rapid than in those poisoned with HN-3 (10). Further injection of H (03) was done before there was remission and resulted in an increase of the severity of the thrombopenia at once, while further injection of HN-3 following remission resulted in the same pattern of thrombopenia and remission as the initial injection. In the dogs poisoned with HN-3 (12) and HN-3 (15) the thrombopenia occurred at about the same time as in those poisoned with smaller doses, but the dogs did not live long enough to show remission.

*Lymph Nodes*—Since the lymph nodes of all the dogs poisoned with vesicants except one were removed at autopsy at least three days after the first injection of the vesicant, the histologic changes which were observed are believed to be secondary to the initial changes produced by the vesicants. In the single dog which died one day after poisoning with HN-3 (15), the mediastinal and mesenteric nodes, appearing grossly as large, dark reddish masses, showed degenerative changes which were markedly different from those observed in nodes of rats at the end of the first day after injection of HN-3 (10). In both dog and rat the lymphocytes of the nodules and general parenchyma showed much karyorrhexis, many degenerated lymphocytes had been ingested by macrophages of reticulum cell origin. In the rat,<sup>1</sup> the nodules at this initial stage showed viable macrophages, and the nodules were full of dying lymphocytes in all stages of karyorrhexis, but in the dog, the nodules were identified only by degenerated remnants of macrophages, and practically no lymphocytes were present. The conditions of cell necrosis and macrophage activity in the parenchyma outside



of the nodules were the same in both rat and dog, but in the dog the general destruction seemed to have been greater than in the rat. In the other degenerative characteristics there was a marked difference between the nodes of the two animals. In the dog, but not in the rat, the peripheral, intermediate and medullary sinuses were congested, and many of them were full of fibrin. As a result of the congestion of the sinuses, the medullary cords were narrowed. In both dog and rat undamaged plasmacytes were present in the medullary cords. In the dog scattered hemosiderin masses in the medullary cords indicated that there had been hemorrhage in this region. Neutrophilic granulocytes were scattered through the congested sinuses of the nodes of the dog.

After these initial changes there were several directions in which the lymph nodes of the poisoned dogs had changed. The most common type of



Fig 1—Photomicrograph of a median longitudinal section of a mesenteric lymph node from dog 1, which died three days after a second injection of 10 mg of tris(2-chloroethyl) amine per kilogram of body weight given forty-five days after the first injection of the same amount of vesicant. Mallory's stain, section, 7 microns thick,  $\times 13$ .

degeneration observed was that in which there was extensive hemorrhage, apparently following the initial congestion. As a result of the hemorrhage the parenchyma was replaced by red blood corpuscles and fibrin. Mesenteric and mesenteric lymph nodes from all 6 dogs poisoned with H, and the nodes from 6 of 9 dogs poisoned with HN-3, showed these hemorrhagic changes. The hemorrhages obliterated the cortex, compressed the medullary cords and filled the sinuses with red blood corpuscles. In these nodes there was no trace of nodules. Remnants of medullary cords containing plasmacytes were present. No granulocytes were observed in any of the nodes. In the nodes

of one of the H poisoned dogs there were areas of bacteria surrounded by necrotic tissue. In most of the nodes, the capsule and the trabeculae were markedly thickened, and there was some fibrosis around the hilus.

The second most characteristic change in the lymph nodes was one in which practically all of the lymphocytes were absent and only the collagenous stroma persisted (fig. 1). The nodes looked as though the lymphocytes had been washed out. The reticulum stroma of the whole lymphatic tissue was beautifully preserved, and the framework of the nodules was particularly well delineated. Scattered reticulum cells remained clinging to the fibers or lying free in the reticulum-lined spaces. The courses of the vasa could be readily traced from the hilus. Despite the absence of lymphocytes, the nodes were not collapsed, a condition probably resulting from the presence of some fibrin in the reticulum spaces. These conditions were the major features in certain of the nodes in 3 of the 6 dogs given injections of H, in the nodes from the dog given HN-1 and in the nodes of 3 of the 9 dogs poisoned with HN-3.

The third type of degeneration might be called a slightly modified continuation of the initial changes. In these nodes there were remnants of the nodules, the parenchyma was loose, but contained small lymphocytes, the medullary cords were well preserved and contained many plasmacytes and there was more or less fibrotic invasion of the cortex and the hilus. The trabeculae and the capsule were usually thicker than normal. Such nodes were present in 3 dogs poisoned with HN-3.

A fourth type of node, characterized by areas of regeneration of the lymphocytes of the cortex, was found in only 1 dog, and it was the one which lived for one hundred and fifty-two days after the initial injection of HN-3 (10). This dog survived four injections and succumbed eighteen days after the fifth injection. Grossly, these nodes were small and white, and there was no fat around them. In the submaxillary and mesenteric lymph nodes of this dog, the capsule and the trabeculae were thicker than normal, and the sinuses were wide and open. The peripheral margin of the cortex was composed of a dense mass of lymphatic tissue, in which many lymphocytes were in mitosis. The medullary cords were composed for the most part of plasmacytes with few lymphocytes. Among these cells were hemosiderin masses, which are believed to be evidence of hemorrhage in this region. A mediastinal node from this same dog showed a smaller amount of regenerating lymphatic tissue, more connective tissue and catarrh of the sinuses.

The histologic conditions observed in the lymph nodes of these poisoned dogs indicate that the initial change is destruction of lymphocytes, accompanied by vascular congestion. The initial changes may be followed by hemorrhage, which prevents regeneration, or by complete washing out of the lymphocytes, leaving no centers for regeneration, or by partial recovery of the lymphatic tissue and regeneration of the lymphocytes. In the lymph nodes of only 1 dog was there evidence of regeneration of the lymphatic tissue, and this dog survived four injections of HN-3. From these facts it would appear that the continued lymphopenia observed in these dogs was caused by the failure of the lymphatic tissue to regenerate, since increase in the number of lymphocytes in the blood occurred only in that dog which showed regeneration in the lymph nodes. Except for this dog, the lymph nodes of the dogs differed from those of the rats in their reaction to the mustard vesicants. In the rats, the secondary changes, such as congestion and hemorrhage, did not occur, and the lymphatic tissue, after its initial intoxication, gave evidence that it could regenerate, and even though the nodes were small, they could still be drained of sufficient lymphocytes to prevent the persistent lymphopenia that occurred in most of the dogs.<sup>1</sup> Hence it is believed that the secondary changes in the lymph nodes, such as congestion and hemorrhage, are

important factors in limiting the production of lymphocytes and in permitting continuance of the severe lymphopenia observed in these dogs

In the lymph nodes of both dog and rat it has been found that the plasmacytes of the medullary cords were remarkably resistant to the poisonous effects of the vesicants. Plasmacytes are usually regarded as descending from lymphocytes and as representing an involutional stage of these cells. In the control dogs the plasmacytes were never so mitotically active as were the lymphocytes. Such conditions suggest that their resistance to the vesicants is caused by the stable condition of their chromatin, as it is known that vesicants and roentgen rays have a selective effect on cells in which the chromatin is in an active condition and do not injure the cells of more stable tissues, such as connective tissue cells, reticulum cells, etc., directly

*Spleen*—The spleen of the dogs poisoned with the vesicants showed when examined grossly evidences of fibrosis of the capsule. The shape of the spleen was modified in only 2 dogs, in one dog it had a large hematoma, and in the other it was deformed from becoming entangled with, and adherent to, the mesentery. Except in 2 dogs poisoned with H (03), in which the relative weights of the spleens were considerably greater than normal, the range of the relative weights of the spleens (from 1.33 to 2.42 Gm per kilogram of body weight) was not significantly different from the range of weights given for 14 thymectomized and 14 control dogs aged from 2 to 16 months (1.03 to 2.64 Gm per kilograms of body weight<sup>5</sup>).

Histologic examination of the spleens of the poisoned dogs showed that all were in a condition of passive congestion, characterized by hyaline collagenous thickening of capsule and trabeculae, open and broken venous sinuses, congestion and fibrosis of the pulp spaces and stasis of the blood in the larger veins of the trabeculae. Examination of the arteries showed that in all the spleens there were atheromatous changes in the walls of the arteries which could have caused the congestion. The larger arteries did not show consistent changes, although the muscle cells of the media of the trabecular arteries were usually infiltrated with fat. But in the smaller arteries the endothelial cells were vacuolated and sloughed into the lumen, the media was markedly infiltrated with fat, and in the ellipsoids the capsule was usually obliterated by hemorrhage. Hence it is believed that the passive congestion present in these spleens was caused by pathologic changes of arteries and capillaries.

Detailed examination of the pulp showed that together with the congestion and fibrous infiltration of the pulp spaces there occurred scattered hemosiderosis, absence of lymphocytes and granulocytes, scattered persistence of reticulum cells, many of which had pyknotic nuclei, and presence of macrophages in variable stages of degeneration with and without hemosiderin. The degenerated cells were not accumulated into masses. Monocytes and plasmacytes were present in some spleens but were never numerous or massed. In several spleens there were areas of necrotic degeneration within which rod-shaped bacteria were observed. These areas infested with bacteria indicate that when pathologic changes occur incident to poisoning with the vesicants, bacterial activity, which might be held in check by the activity of surrounding tissues receiving a normal blood supply, may increase in virulence when this supply is cut off by the degenerative changes produced in the vasa by the vesicants. The presence of these bacteria also implies that *in vivo* the vesicants are not bactericidal.

In addition to the congestive degeneration, the spleen showed marked evidence of degenerative changes in the lymphoid tissue. The malpighian corpuscles of the spleens of all dogs showed definite evidence of degeneration. This degeneration

in the lymphoid nodules was characterized by small size, looseness, lack of medium-sized lymphocytes, pyknosis of the nuclei of small lymphocytes and of many of the reticulum cells, fragmentation of the cytoplasm of the reticulum cells and macrophages, much edema, hemorrhage within the center and around the margins of the nodules and absence of mitosis in the cells present. The congestion could have caused the hemorrhage and the reduction in the size of the nodules, but the direct action of the toxic vesicants is presumed to have damaged the lymphocytes before the secondary effects occurred. The nodules appeared to be in such a degenerated condition that there seemed little chance that they would recover activity and resume production of lymphocytes.

The lymphoid cords surrounding the arteries of the pulp were markedly reduced in amount in the spleens of all the dogs. The reticulum cell stroma remained, and within its meshes there were a few lymphocytes, usually with pyknotic nuclei, and some plasmacytes. The cords were usually separated from the surrounding pulp by flattened reticulum cells and fibers. The tissue was free of hemorrhage, but in some places it contained masses of hemosiderin or macrophages with hemosiderin.

The volume of the lymphoid tissue, including the malpighian corpuscles, which in sample sections of the spleens of the normal dogs amounted to about 44 per cent of the total volume, was lower in the spleens of all the poisoned dogs. The volume of this tissue was least in the dogs given injections of H (range, 07 to 30 per cent) and in the dogs poisoned with the greatest amounts of HN-3 (range, 08 to 11 per cent). Taking into consideration these data and those from the qualitative study, one may conclude that the vesicants not only damaged the cells but reduced the amount of lymphoid tissue.

The pathologic changes in the spleens of these dogs poisoned with vesicants were much greater than those which occurred in the rats poisoned with the same vesicants in about the same dosage.<sup>1</sup>

*Tonsils*—None of the histologic features characteristic of the normal tonsil were seen in sections of the tonsils of the dogs poisoned with vesicants. Unfortunately, the tonsils were not taken from all the dogs, but sufficient material was obtained to give an idea of the changes which followed the injection of the vesicants. Initially, as in the lymph nodes, the poisoning was followed by destruction and phagocytosis of the lymphocytes, obliteration of the topography of the nodules and congestion of the vasa. This is a picture of cell destruction and inflammation. Such a condition is usually followed by involution, characterized by further reduction in the amount and the regular arrangement of the lymphoid nodules, dissolution of the dead phagocytes, thinning of the epithelium, increase of the amount of connective tissue and development of plasmacytes. Plasmacytes were occasionally seen in the normal tonsil, but they never formed layers and sheets such as were present beneath the epithelium of the tonsils of the poisoned dogs. These conditions usually occurred by the third day after poisoning. If the dog survived for a longer period, the tonsils shrank, the connective tissue increased, the epithelium became thicker, and the remnants of the lymphoid tissue began to undergo regeneration. The masses of regenerating lymphocytes were composed of mingled reticulum cells and lymphocytes. These masses lay near the longitudinal axes of the tonsils. Peripheral to the masses were regions characterized by large numbers of plasmacytes, which permeated the fibrotic stroma. It appeared that as the lymphocytes were produced they became modified into plasmacytes, since there was no evidence of local proliferation of the plasmacytes.

The lymphocytes of the tonsils were initially poisoned by the vesicants, as were those of the lymph nodes and the spleen, but the subsequent changes in the tonsils differed from those in both lymph nodes and spleen in the absence of hemorrhage. The tonsils were further different from the lymph nodes in retention of lymphocytes and in a tendency to produce large numbers of plasmacytes in the region formerly occupied by the nodules, whereas in the lymph nodes the plasmacytes, while persisting, were confined to the medullary cords.

*Thymus*—In the normal adult dog the thymus is in a condition of involution<sup>4</sup> and does not contribute to the lymphocyte population of the blood.<sup>5</sup> In the dogs given injections of vesicants the thymus when present was in a condition of involution characterized by shrunken lobules composed of a loose reticulum stroma containing few lymphocytes, hydropic macrophages and congested vasa. The lobules were separated by edematous connective tissue. In some of the dogs there was no trace of the thymus. It is possible that the vesicants had speeded up the process of involution as they do in the rat, but the changes in the structure of the thymus apparently had nothing to do with the lymphopenia of the peripheral circulation.

*Bone Marrow*—The marrows of the ribs and the sternum of 4 young dogs served as controls for conditions in the bone marrows of the poisoned dogs. Stained sections of marrow were used for histologic study and for making counts of cells per 500,000 cubic microns. The percentage distributions of the different types of cells were determined from counts of 500 cells from each region of each dog in touch smears, dried and stained by the May-Giemsa method. In a few of the poisoned dogs the marrows of femur and vertebrae were studied. The numbers of different types of cells per unit volume (500,000 cubic microns) were calculated from these data and are presented in table 4. Myeloid and erythroid cells in mitosis per 25 unit volumes (50,000 cubic microns per unit volume) were counted from each region of the sectioned material.

In the sternal marrow of the control dogs, the cells were closely packed, and there were few open sinuses and little fat (fig 2). The average number of cells per unit volume in the sternal and rib marrow was  $1,960 \pm 82$  (table 4). The myeloid-erythroid ratio in the sternum was 0.9:1.0, that in the ribs, 1.5:1.0. The sternal marrow was less myeloid than that of the dogs studied by Alexandrov<sup>6</sup>, the ratio in the rib marrow agreed with that of Mulligan<sup>7</sup> on young dogs, but was less myeloid than the marrow of the dogs studied by Stasney and Higgins<sup>8</sup>. Since the cells in the marrow of the poisoned dogs were so few, the separate classes of myeloid and erythroid cells have not been entered as such in table 4, but all of the immature myeloid cells and erythroid cells have been classified as myeloid and erythroid cells, respectively. Cells which had little or no representation in the normal marrow but were present in the marrow of the poisoned dogs included degenerated myeloid cells, macrophages with hemosiderin debris, and plasmacytes.

In the bone marrow of all the poisoned dogs at death there was cellular hypoplasia (fig 2, B). There was considerably more fat in the marrow of these dogs than in that of the controls. In most of the dogs the marrow was hyperemic, and the surviving cells were scattered between the fat and the congested sinusoids.

4 Park, E. A., and McClure, R. D. *Am J Dis Child* **18** 317, 1919.

5 Hammar, A. *Endocrinology* **5** 543, 1921.

6 Alexandrov, A. F. *Folia haemat* **41** 428, 1930.

7 Mulligan, R. M. *Anat Rec* **79** 101, 1941.

8 Stasney, J., and Higgins, G. M. *Am J M Sc* **193** 462, 1937.

Fibrin deposits in places formerly occupied by cells were common. There were no islets of erythroid cells even in the marrow of those dogs in which these cells survived in greater numbers than in others in which they were few. The numbers of megakaryocytes were greatly reduced.

Qualitatively the myeloid cells which were present showed many signs of degeneration, such as vacuolated nuclei and cytoplasm, coarseness of chromatin, swelling of the nuclei and deficiency of granules in the cytoplasm. In some dogs, particularly those poisoned with HN-3, there were large numbers of myeloid cells which were counted as degenerated and in which there seemed to be cessation of growth and differentiation of the cytoplasm. Macrophages with hemosiderin and other debris were more abundant in the poisoned dogs than in the controls (table 4). In the myeloid cells mitosis was suppressed, and in most of the marrows there was no evidence of regeneration. There was practically no mitosis in the erythroid cells. The incidences of erythroid cells were reduced practically to zero in the H poisoned dogs, but showed greater degrees of survival in those

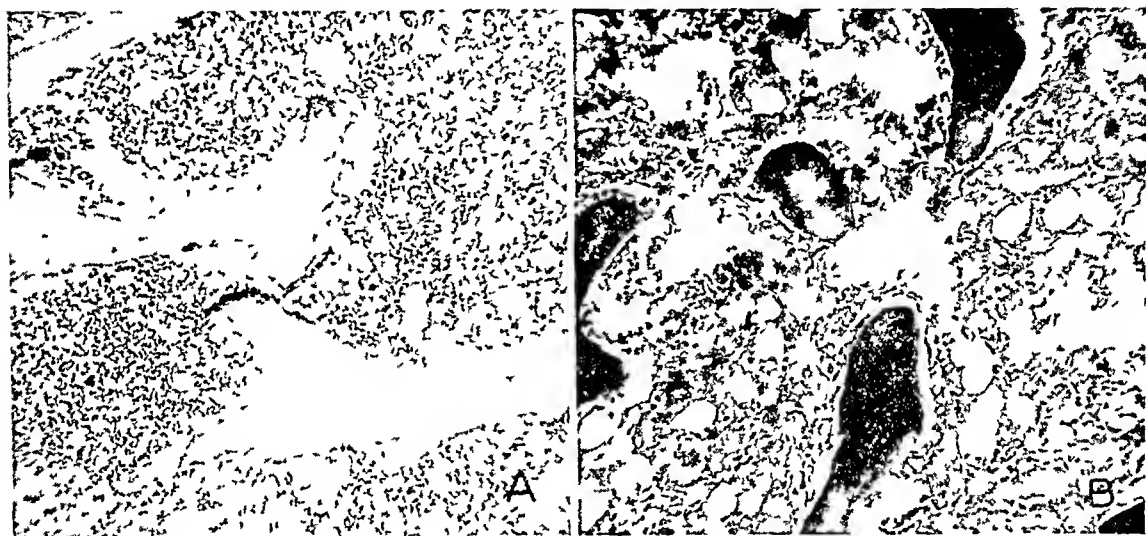


Fig 2—Photomicrographs of sections of marrow of the sternum. *A*, marrow from a control dog, hematoxylin and eosin, section, 5 microns thick,  $\times 90$ . *B*, marrow from dog 7, which died four days after injection of 10 mg of bis(2-chloroethyl) sulfide per kilogram of body weight, Mallory's stain, section, 5 microns thick,  $\times 90$ .

poisoned with HN-1 or HN-3 (table 4). The greatest survival of erythroid cells occurred in the HN-3 poisoned dogs. Myelocytes were not as uniformly reduced in number as were the erythroid cells, and the greatest relative survival of myelocytes was in the dog poisoned with HN-1 (table 4).

A quantitative study of the sizes of the myelocytes in the poisoned dogs in contrast with those in the controls showed that there was no significant swelling of the myelocytes in the H poisoned dogs but that there was in the dog poisoned with HN-1 and in one of the dogs poisoned with HN-3.

First, if it is assumed that in the dog the life tenure of the neutrophilic granulocytes is about four days, as it has been estimated to be in the rabbit,<sup>9</sup> then if the dogs survived poisoning for four days and there was a condition of extreme

9 Weiskotten, H. G. *Am J Path* 6:183, 1920.

TABLE 4.—Bone Marrow Effects of Intravenously Injected Bis(2-Chloroethyl) Sulfide (H), Ethyl-Bis(2-Chloroethyl) Amine (HN-3),<sup>1</sup> and Tris (2-Chloroethyl) Amine (HN-3)<sup>1</sup>

| Dog | Control | Dose, Mg per kg of Body Wt | Visceral | Days Injections | Elapsing | Total Number of Cells | Myelocytes | Neutrophilic Myelocytes | Eosinophilic Myelocytes | Degen-erated Myelocytes | Meta-rubricytes | Reticulum Cells | Macro-phages | Lympho-cytes | Plasma-cytes |
|-----|---------|----------------------------|----------|-----------------|----------|-----------------------|------------|-------------------------|-------------------------|-------------------------|-----------------|-----------------|--------------|--------------|--------------|
| 11  | H       | 0.3                        |          | 1               | 7        | 1,960 ± 82            | 700 ± 65   | 138 ± 18                | 86 ± 11                 | 0                       | 790 ± 54        | 39 ± 8          | 0            | 91 ± 17      | 0            |
|     |         | 0.3                        |          | 2               | 5        | 132 ± 29              | 1 ± 1      | 1 ± 0                   | 0                       | 0                       | 1 ± 1           | 11 ± 0          | 7 ± 2#       | 48 ± 11      | 57 ± 15#     |
| 12  | H       | 0.3                        |          | 1               | 7        | 450 ± 100             | 121 ± 21   | 3 ± 1                   | 1 ± 1                   | 1 ± 1                   | 42 ± 9          | 72 ± 54         | 14 ± 2#      | 62 ± 1       | 101 ± 9#     |
|     |         | 0.3                        |          | 2               | 5        |                       |            |                         |                         |                         |                 |                 |              |              |              |
| 9   | H       | 0.3                        |          | 1               | 5        | 172 ± 9               | 3 ± 1      | 2 ± 0                   | 1 ± 1                   | 1 ± 0                   | 3 ± 1           | 79 ± 6#         | 24 ± 0#      | 20 ± 1       | 33 ± 3#      |
| 10  | H       | 0.5                        |          | 1               | 4        | 130 ± 49              | 4 ± 1      | 2 ± 0                   | 1 ± 1                   | 2 ± 1                   | 8 ± 6           | 55 ± 22         | 20 ± 7#      | 23 ± 6       | 18 ± 3#      |
| 7   | H       | 1.0                        |          | 1               | 4        | 110 ± 26              | 8 ± 4      | 3 ± 0                   | 1 ± 1                   | 1 ± 1                   | 2 ± 1           | 53 ± 7          | 10 ± 2#      | 7 ± 0        | 21 ± 5#      |
| 8   | H       | 1.0                        |          | 1               | 4        | 90 ± 43               | 11 ± 2     | 2 ± 1                   | 1 ± 0                   | 1 ± 1                   | 9 ± 6           | 21 ± 6          | 5 ± 3        | 11 ± 6       | 25 ± 24      |
| 6   | HN 1    | 1.0                        |          | 1               | 6        | 410 ± 20              | 240 ± 0    | 53 ± 5                  | 5 ± 4                   | 30 ± 3#                 | 30 ± 0          | 26 ± 17         | 6 ± 1#       | 1 ± 1        | 5 ± 0#       |
| 1   | HN 3    | 1.0                        |          | 1               | 45       | 175 ± 40              | 17 ± 16    | 2 ± 2                   | 6 ± 6                   | 43 ± 7#                 | 29 ± 28         | 13 ± 0          | 9 ± 8        | 7 ± 5        | 44 ± 33      |
|     |         | 1.0                        |          | 2               | 3        |                       |            |                         |                         |                         |                 |                 |              |              |              |
| 14  | HN 3    | 1.0                        |          | 1               | 14       | 107 ± 27              | 1 ± 1      | 1 ± 0                   | 1 ± 0                   | 0                       | 33 ± 4          | 4 ± 2           | 4 ± 1#       | 36 ± 5       | 0            |
|     |         | 1.1                        |          | 2               | 27       |                       |            |                         |                         |                         |                 |                 |              |              |              |
|     |         | 1.1                        |          | 3               | 3        |                       |            |                         |                         |                         |                 |                 |              |              |              |
| 2   | HN 3    | 1.0                        |          | 1               | 45       | 325 ± 50              | 172 ± 50   | 19 ± 6                  | 1 ± 1                   | 50 ± 34                 | 57 ± 3          | 14 ± 1          | 1 ± 0        | 2 ± 0        | 0            |
|     |         | 1.0                        |          | 2               | 40       |                       |            |                         |                         |                         |                 |                 |              |              |              |
|     |         | 1.0                        |          | 3               | 20       |                       |            |                         |                         |                         |                 |                 |              |              |              |
|     |         | 1.1                        |          | 4               | 23       |                       |            |                         |                         |                         |                 |                 |              |              |              |
|     |         | 1.1                        |          | 5               | 18       |                       |            |                         |                         |                         |                 |                 |              |              |              |
| 4;  | HN 3    | 1.2                        |          | 1               | 9        | 288 ± 56              | 71 ± 5     | 8 ± 5                   | 2 ± 0                   | 6 ± 2 4                 | 158 ± 47        | 15 ± 1          | 3 ± 2        | 2 ± 1        | 3 ± 1#       |
| 15  | HN 3    | 1.5                        |          | 1               | 4        | 91 ± 4                | 1 ± 1      | 1 ± 0                   | 0                       | 0                       | 0               | 48 ± 1          | 3 ± 1        | 7 ± 1        | 31 ± 5#      |
| 18  | HN 3    | 1.5                        |          | 1               | 4        | 192 ± 8               | 1 ± 0      | 1 ± 1                   | 1 ± 1                   | 40 ± 22                 | 3 ± 2           | 93 ± 17#        | 11 ± 1#      | 8 ± 2        | 27 ± 10#     |

\* The table shows the distribution of the cells per 500,000 cubic microns of marrow of sternum and ribs (unless otherwise indicated) of the poisoned dogs as contrasted with conditions in 4 normal dogs. The total numbers of cells were counted in sections, and the differential counts were made from smears in which 500 cells were counted from each dog. Each number is accompanied by the standard error of its mean. All numbers except those of the control which are not marked by the symbol # or the symbol || are significantly less than the control mean.

† This column gives the days elapsing between injections or from the time of the first injection to death.

‡ Marrow of a femur was used in addition to that of ribs and the sternum.

§ Marrow of a femur was used instead of that of the sternum.

|| The number is not significantly different from the control mean.

# The number is significantly larger than the control mean.

neutropenia in the peripheral blood, the marrow may be assumed to have been poisoned to such a degree by the vesicant that it could effect no replacement of neutrophils. Under these conditions there might be myeloid cells in the marrow, but they would remain unproductive. Of 5 dogs poisoned with H, there was only 1 (no 12, table 4) which would fall into this category, the only dog poisoned with HN-1 (no 6, table 4) and 2 dogs (nos 2 and 4, table 4) of 6 poisoned with HN-3 would also be included. Second, if the peripheral blood was agranulocytic and the bone marrow was without myeloid cells at the same time, it may be assumed that, in addition to the normal loss of the granulocytes of the peripheral blood, the myeloid cells of the marrow were destroyed by the poison and that there would be no possibility of regeneration. This condition was present in 4 out of 5 dogs poisoned with H and in 1 of the 6 poisoned with HN-3. Third, if there was a moderate diminution of the granulocytes in the peripheral blood, practically no myelocytes in the bone marrow, and the dogs died before the fourth day after the first injection, or a further injection, it may be assumed that the myelocytes of the bone marrow were destroyed, and that as soon as the normal loss of granulocytes occurred there would be no replacement. This condition was present in 3 of the 6 dogs poisoned with HN-3, and 2 of these were poisoned with the greatest amounts of HN-3 (nos 15 and 18, table 4). From these analyses it will be seen that H has a greater degenerative effect on the myeloid cells than HN-1 or HN-3 but that HN-3 is more toxic than HN-1.

Using these data for prognosis, one may, it is suggested, make a prognosis unfavorable for survival if the granulocytes of the peripheral blood continue to decrease sharply without replacement after the fourth day of injection. If, however, the neutropenia does not become extreme by the fourth day and begins to be alleviated by the seventh day, the prognosis is favorable. It is also of interest to note that in those dogs in which myelocytes were present in the marrow at death, there was a significant shift to the left of the immature neutrophils just before death or on the day of death. Thus a shift to the left of neutrophils could possibly be prognostic of impending death, provided there was marked neutropenia of the blood at the same time.

There was a consistent relation between the incidences of lymphocytes in the marrow and in the peripheral blood of the poisoned dogs. In the H poisoned dogs, which had higher peripheral lymphocyte counts than the dogs poisoned with other vesicants, there were more lymphocytes in the marrow (table 4). Relative hyperplasia of plasmacytes was consistently present in the H poisoned dogs, occurred to a slight degree in the HN-1 poisoned dog and was of variable occurrence in the HN-3 poisoned dogs (table 4). These plasmacytes appeared to be derived from the lymphocytes and not from reticulum cells.

There seemed to be no consistent relation between the incidence of lymphocytes and the incidence of the myeloid or the erythroid cells in the bone marrow. If it is assumed that normally lymphocytes are drained off into the marrow to serve as ancestors for the myeloid and erythroid cells as hemoblasts,<sup>10</sup> it would be expected that in the marrows of those dogs with the greatest numbers of lymphocytes there would be consistently higher levels of myeloid and erythroid cells, but in the samples studied there is no such relation (table 4).

In all the dogs there was extreme hypoplasia of the erythroid cells in the marrow (table 4). The erythroid cell hypoplasia was relatively much greater than the anemia of the peripheral blood no matter how long the dogs had lived or how many injections they had received. The initial anemia in the peripheral

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10 Jordan, H. E. *Anat. Rec.* **73** 227, 1939.



blood of the dogs poisoned with H or HN-3 was about what would be expected if the proliferation of the erythroid cells of the whole marrow had been inhibited by the vesicant immediately after injection and there was no replacement of the cells normally lost at the rate of 0.75 per cent of the red blood corpuscles per day with a life tenure for the red blood corpuscles of the dog of about one hundred and twenty-four days<sup>11</sup> However, in the HN-1 poisoned dogs the anemia was so severe on the second day after injection as to suggest that not only had the poisoning caused inhibition of production but also greater than normal loss of red blood corpuscles of the peripheral blood The hypoplasia of the erythroid cells in the marrow of the dogs which died at the end of four days after poisoning—e g, no 8 (H, 10) and nos 15 and 18 (HN-3, 15)—was of such a degree that the destruction of these cells should have shown greater decreases in the numbers of red blood corpuscles in the peripheral blood than was observed Since no decreases occurred before death, it must be assumed that there are other factors which maintained the numbers of red blood corpuscles despite the poisoning of the erythroid cells of the marrow The fact that the spleen, which is normally full of red blood corpuscles, was practically ischemic in all of these dogs leads to the inference that the withdrawal of the blood from the spleen would account for the maintenance of the numbers of red blood corpuscles in the peripheral blood When the dogs survived poisoning for more than four days, the numbers of red blood corpuscles began to decrease, and such decreases could be accounted for by the hypoplasia of the erythroid cells of the bone marrow and their slow regeneration between injections Finally, as a result of continued poisoning, the regeneration of the erythroid cells was slowed, and although the conditions of anemia imposed by the initial injection did not become more severe, there was no remission Every time a dog was given another injection, the anemia became aggravated, because there was no longer a reserve of red blood corpuscles in the spleen and more erythroid cells in the marrow were poisoned Furthermore, judging from the small numbers of erythroid cells present in the marrow of the poisoned dogs at death, one concludes not only that the proliferation was inhibited but that most of the cells were destroyed HN-1 apparently has a greater immediate destructive effect than H or HN-3, but the extent of terminal destruction may be of the same degree after poisoning with any of the vesicants The lack of correlation between the numbers of red blood corpuscles in the peripheral blood and the decrease of the numbers of erythroid cells in the marrow resembles closely the conditions found in man treated with therapeutic doses of methyl-bis(2-chloroethyl) amine in whom the quantitative distributions of the cells of the sternal marrow were followed by biopsy<sup>12</sup>

In all of the dogs in which the marrow was studied there was thrombopenia at death In those which had only one injection of lethal doses of H, there was moderate to severe thrombopenia, and there were practically no megakaryocytes in the samples of marrow studied In those which had more than one injection of H, the same condition obtained, and they never recovered from the initial poisoning of the megakaryocytes In the dogs poisoned with HN-1 there was severe thrombopenia at death and despite the persistence of myeloid and erythroid cells in the marrow, there were no megakaryocytes Dogs poisoned with the strongest doses of HN-3 in which there was moderate thrombopenia in the blood had some megakaryocytes in the very hypoplastic marrow In 1 dog which had five

11 Hawkins, W B, and Whipple, G H *Am J Physiol* **122** 418, 1938

12 Spurr, C L, Jacobson, L O, Smith, T R, and Barron, E S G, in Moulton,<sup>2</sup> p 24

injections of HN-3 there was thrombopenia at death although megakaryocytes were still present in the marrow. The persistence of the megakaryocytes probably accounted for the recovery of this dog from the thrombopenia which followed each injection. From these data it would appear that H and HN-1 are more toxic to the megakaryocytes of the marrow than is HN-3 and that the toxic effects are directly related to the thrombopenia of the circulating blood.

*Adrenal Gland*—Quantitative and qualitative histologic studies were made of the adrenal glands of the dogs poisoned with vesicants and the conditions contrasted with those observed in the adrenal glands of nonpoisoned dogs. The average relative weight of the glands did not show any significant changes from the average normal relative weight of 0.117 Gm per kilogram computed from Baker's<sup>11</sup> values.

The adrenal glands of the poisoned dogs all showed evidence of pathologic changes, which appeared to be secondary to damage done to the blood vessels. The changes which have been analyzed quantitatively showed hyaline thickening of the capsules, decreases in the nuclear and cytoplasmic volumes of the cells of the cortex and decreases in the amount of chromaffin tissue in the medulla. The nuclei of the cells of the medulla were less affected by the poisons than were those of the cells of the cortex. Cell proliferation was practically inhibited, so that there was little possibility of regeneration. Focal necrosis, hemorrhage and congestion were particularly characteristic of the zonae fasciculata and reticularis. Taking all the pathogenic effects into account one concludes that HN-3 had the most intoxicating effect on the cells of the cortex. H and HN-1 had about the same effect. H, however, had the most intoxicating effect on the medulla and HN-1 the least.

Pathologic changes such as have been observed in the adrenal glands of the dogs poisoned with vesicants were not found in the glands of rats poisoned with the same relative amounts of these vesicants.<sup>1</sup> In the rats which survived for four days there was moderate hypertrophy of the cells and enlargement of the vacuoles in the spongy zone of the zona fasciculata. There was some congestion in the zona reticularis, but it was never accompanied by degeneration of cells. Ludewig and Chanutin<sup>14</sup> have shown that in the adrenal glands of such rats the total cholesterol concentration and the total lipid concentration are decreased. Therefore, there seems to be a correlation between the swelling of the cells and the decrease of the substance which may be the basis for the production of the cortical hormone. In the poisoned dogs, the cells in the spongy zone are smaller than in the controls, the vacuoles are enlarged, and there is much focal necrosis and pathologic change in this zone. From a contrast between the histologic aspects of the cortices of the glands of the poisoned rats and dogs the inference is made that the production of the cortical hormone is diminished to a greater degree in the dog than in the rat.

Other investigators who have studied the adrenal glands of guinea pigs intoxicated with sulfur mustard (H),<sup>15</sup> phenol, chloroform, carbon tetrachloride, dichloromethane and tetrachloromethane<sup>16</sup> have reported pathologic changes similar in general to those observed in the adrenal glands of the poisoned dogs. Both Graham<sup>16</sup> and Hoerr<sup>15</sup> have emphasized the fact that the zona reticularis is the site of the earliest and most extensive lesions. Similar pathologic changes

13 Baker, D. D. *Am J Anat* **60** 231, 1937

14 Ludewig, S., and Chanutin, A. *Endocrinology* **38** 376, 1946

15 Hoerr, N. *Am J Anat* **48** 139, 1931

16 Graham, G. S. *J M Research* **34** 241, 1916

have been found in the adrenal glands of dogs in which the suprarenal vein was ligated<sup>17</sup> These dogs showed the same symptoms of general weakness, disturbances of alimentation, etc., as were observed in the dogs poisoned with vesicants

Since these pathologic changes occurred in the adrenal glands of dogs which had at the same time suffered widespread intoxication of the lymphoid organs, it is believed that the cortical hormone is not responsible for the initial damage of the lymphocytes These data support the view expressed earlier after a study of the effects of the vesicants on adrenalectomized rats in which intoxication of lymphocytes occurred in the same manner as it did in nonadrenalectomized rats which had been given injections of vesicants<sup>1</sup>

*Other Organs*—No other organs of the poisoned dogs were taken systematically for histologic study, but sample sections were made from some organs which appeared on gross examination to differ from the normal Among these organs were the small intestine, the cecum, the colon, the liver, the pancreas and the kidney In 2 dogs which had been poisoned with several injections of HN-3, either the epithelium of the intestinal villi was sloughed or the cells were hypertrophied, as were the cells which had been exposed to roentgen rays<sup>18</sup> There were no mitoses in the cells of the glands, a condition which would prevent the normal rapid replacement of the epithelial cells of the villi<sup>19</sup> In the poisoned rats damage to this part of the intestine it was inferred, offered an avenue for greater loss of blood cells than occurs in the normal animal and would thus contribute to the leukopenia and anemia observed in the peripheral blood<sup>1</sup> That the same conditions occurred in the dog lends support to this view and may possibly account for the rapid decrease of the lymphocytes of the blood during the first five hours after injection of the vesicant even though the cells are delivered in normal numbers to the blood via the thoracic duct during this period<sup>20</sup> Not only is it likely that blood is lost through the damaged intestine but it is obvious that material needed in the production of blood cells cannot be normally absorbed from ingested food The cecum and colon of 2 dogs poisoned with HN-3 showed sloughing and coagulation necrosis of the mucosa The livers of 2 dogs poisoned with H (03) were completely necrotic, and in one of them bacteria were present In the liver of the dog poisoned with repeated injections of HN-3 there was extensive portal cirrhosis The pancreas of this dog showed patchy degeneration of the acini and shrinkage of the islands of Langerhans The right kidney of this dog was only a cystic sac, and the left showed evidences of acute glomerulonephritis with patchy necrosis

In these dogs, particularly those poisoned with the greatest amounts of the vesicants, there were decrease in the albumin and increase in the alpha globulin fraction of the serum,<sup>3</sup> as well as increases in the plasma fibrin and cholesterol<sup>4</sup> The latter condition was particularly marked in the dogs given repeated injections of H (03), in which there was evidence also of necrosis of the liver

Observations on the ganglion cells of ganglions of the autonomic nervous system near the adrenal gland indicated that the cells had been damaged In four ganglions studied there were all degrees of degeneration of cells, primarily of the nucleus and secondarily of the cytoplasm The nucleolus appeared to be swollen in most of the cells and in several cells it was observed in a position suggesting that it was being extruded through the nuclear membrane The injured nucleus was wrinkled, the nuclear membrane had blunt projections and was chromatic The injured

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17 Rogoff, J M Am J Path **38** 392, 1944

18 Warren, S, and Friedman, N B Am J Path **18** 499, 1942

19 Leblond, C P, and Stevens, C E Anat Rec **100** 357, 1948

20 Courtice F C, and Jones, R P Unpublished data, 1944

cytoplasm was dense around the nucleus and vacuolated at the periphery, and the cell membrane was indistinct. The capsule and neurilemma cells were chromatic, and the capsules were broken. Such conditions could be a result of congestion and anoxia incident to some process interfering with the blood supply or they could have been caused directly by the vesicant.

These meager observations are presented to suggest that while the vesicants are primarily poisoners of the hemopoietic cells, they also have general toxic effects. From the relations of the pathologic effects it appears that the degeneration observed was secondary to injury of the blood vessels, particularly to that of the capillaries. It is possible that the degeneration observed in the hemopoietic organs is also related to the damage of the capillaries, since changes occur in organs which have extensive capillary nets and the circulating poisons would have had immediate access to the hemopoietic cells if the wall of the capillary was injured.

#### COMMENT

The descriptive data presented in this paper support the views of various investigators<sup>21</sup> that the beta chloroethyl vesicants exert a selective toxic action on the nuclei of actively proliferating cells of the lymphoid organs and bone marrow in mammals. The results of this action were observed in the quantitative decreases in the cells of the peripheral blood of the poisoned dogs. The extent of cellular intoxication was directly related to the amount of vesicant injected in a manner such as that reported by Karnofsky and associates.<sup>22</sup> With sublethal doses of the vesicants, repeated injection of the dose produced the same pattern of change in the peripheral blood as did the initial injection. The leukopenia of the peripheral blood followed the same pattern as was observed in the victims of the atomic bomb radiation in Japan<sup>23</sup> and in animals exposed to the atomic bomb radiation in test "Able" at Bikini.<sup>24</sup> Also the skeletonized appearance of some of the lymph nodes of the poisoned dogs, consisting largely of reticulum stroma, cell debris, fibrin and macrophages, was like that characterizing the human victims. Similar, too, were the hypoplasia of the bone marrow and the pathologic changes in the spleen. In gross pathologic aspects the lymph nodes and the marrow, but not the spleen, were similar to the same organs of the animals exposed at Bikini.

In contrasting the morphologic aspects of the lymph nodes of rats<sup>1</sup> and dogs which were killed or died at the same time after injection of the same relative amounts of the vesicants, the nodes of the poisoned dogs showed greater pathologic changes and greater loss of lymphocytes than did those of the rats. This greater pathologic effect of the vesi-

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21 Gilman, A., and Philips, F. S. *Science* **103** 409, 1946. Kindred,<sup>1</sup> Moulton.<sup>2</sup>

22 Karnofsky, D. A., Burchenal, J. H., Ormsbee, R. A., Cornman, I., and Rhoads, C. P., in Moulton,<sup>2</sup> p. 11.

23 Le Roy, G. V. *J. A. M. A.* **134** 1143, 1947.

24 Tullis, J. L., and Warren, S. *J. A. M. A.* **134** 1155, 1947.

cants on the dog than on the rat is also noted with respect to the spleen, the bone marrow, the small intestine, the liver and the adrenal glands. The present findings indicate that not only are the vesicants rapidly acting specific poisons, but they have a slower, more general intoxicating effect. This effect seems to be associated with damage of the capillaries, since in the lymph nodes, the spleen and the adrenal glands there is evidence of sloughing of the endothelium, congestion and hemorrhage. These pathologic changes occurring in the capillaries are particularly damaging to organs with rich blood supply, and it is possible that they caused the cystic degeneration found in the kidney of one of the dogs repeatedly poisoned with HN-3. The secondary pathologic changes, while not influencing the blood picture directly, may produce conditions in the lymph nodes and the spleen which prevent adequate transport of such materials as may be absorbed from the damaged small intestine and which are necessary for the adequate metabolism of the whole animal.

With sublethal doses the damage produced by the vesicants is largely on the proliferative cells, and judging from the regenerative changes in the blood, one concludes that the injured hemopoietic centers can regenerate. Myeloid cells start to regenerate within eight or nine days after the initial poisoning, and the regeneration is indicated by a shift to the left of the neutrophilic granulocytes of the blood, but the lymphocytes, although showing slight regenerative capacities during the second week, never reach such relatively high values as do the neutrophils. The thrombocytes follow about the same pattern of regeneration as do the neutrophils. With lethal doses the damage to both the proliferative cells and to the organ structure, particularly the capillaries, is such that regeneration is slow or does not take place. Extensive pathologic changes in the lymphoid organs are often accompanied by bacterial invasion and necrosis of tissue not damaged by the agents. Such bacterial invasion has been seen in dogs in which normal flowing of lymph through the lymph nodes has been blocked<sup>25</sup>. Furthermore, the vesicants have been shown to prevent antibody formation,<sup>26</sup> the absence of which may account for the observed bacterial invasion of the lymphoid organs of the poisoned dogs.

Another reaction which has been observed in the poisoned dogs and which remains unexplained is the relative hyperplasia of the plasma cells in the bone marrow at the time when the myeloid and erythroid cells have decreased in number. The plasma cells appear to be derived from lymphocytes and not from reticulum cells as they are in the bone

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<sup>25</sup> Drinker, C. K., Field, M. E., Ward, H. K., and Lyons, C. *Am. J. Physiol.* **112** 74, 1935.

<sup>26</sup> Spurr, C. L. *Proc. Soc. Exper. Biol. & Med.* **67** 259, 1948.

marrow of rabbits after anaphylactic shock<sup>27</sup> Plasma cell hyperplasia has also been found in the hypoplastic bone marrow of the victims of atomic bomb radiation<sup>23</sup> Regardless of their origin, the plasmacytes are always present in the hypoplastic marrow and seem to be formed in greater numbers when there is no accumulation of degenerated cells

Aside from the secondary pathologic effect of the vesicants on the hemopoietic organs, the pathologic changes observed in the adrenal glands could partially account for the somatic and visceral conditions of general debility, muscular weakness, etc., present in the poisoned dogs at death, since both the symptomatic conditions and the pathologic changes of the adrenal glands resemble those of dogs in which the veins of the adrenal glands have been ligated<sup>17</sup>

The observations made in this report of the degenerative changes in the nerve cells of the autonomic ganglions in the poisoned dogs lend anatomic support to the pharmacologic observations that one of the cyclic derivatives produced by the hydrolysis of the 2-chloroethyl vesicants has a paralytic effect<sup>28</sup> If great numbers of nerve cells should be damaged, it would appear that the abnormal physiologic conditions observed in the alimentary systems of the poisoned dogs could be attributed to such damage

Unfortunately, at the present time there does not seem to be an agreement as to how the initial nucleotoxic effect of the 2-chloroethyl vesicants is produced<sup>29</sup> Selective physical damage is done to the cells which react with tagged phosphorus,<sup>30</sup> to cells that contain high concentrations of desoxyribose nucleic acid,<sup>31</sup> to cells whose membranes are made more permeable by roentgen rays *in vitro*<sup>32</sup> and to cells which contain a physically demonstrable content of an alkaline phosphatase in the nucleus<sup>33</sup> Poisoning could occur directly through the blocking of the enzyme systems necessary for the high metabolic activity incident to mitosis The various possibilities for selective reaction have been discussed at length by Gilman and Philips,<sup>29</sup> but the explanation of the reaction is still in doubt

#### SUMMARY

Daily counts of white and red blood corpuscles and thrombocytes in dogs poisoned with varying amounts of bis(2-chloroethyl) sulfide and the hydrochlorides of ethyl-bis(2-chloroethyl) amine and tris(2-chloro-

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27 Good, R A Proc Soc Exper Biol & Med **67** 203, 1948

28 Philips, F S, and Gilman, A, in Moulton,<sup>2</sup> p 3

29 Philips and Gilman<sup>28</sup> Gilman and Philips<sup>21</sup>

30 Andreasen, E, and Ottensen, J Acta path et microbiol Scandnav, 1944, supp 54, p 25

31 Gjessing, E C Federation Proc **7** 156, 1948

32 Schrek, R J Cell & Comp Physiol **28** 277, 1946

33 Wislocki, G B, and Dempsey, E W Anat Rec **96** 249, 1946

ethyl) amine showed significant relations between the neutropenia, lymphopenia, anemia and thrombopenia observed in the peripheral blood and the hypoplasia of cells and inhibition of mitosis observed in the lymph nodes, the spleen and the bone marrow

Secondary pathologic changes were present in all of these organs, and these are believed to have interfered with the proper functioning of these organs and to have imposed conditions which prevented normal regeneration. In addition, pathologic changes were present in the adrenal glands, small intestine, cecum, colon, liver, pancreas, kidneys and tonsils which are thought to have been caused by the damage done to the capillaries and which contributed to the general conditions of intoxication which occurred in these dogs

The hemopoietic organs of the dog seem to be more susceptible to the damage caused by the 2-chloroethyl vesicants than are those of the rat

## EFFECT OF ANTI-RAT-LIVER SERUM ON RATS

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CHICAGO

THE ROLE of specific tissue sensitivity as a factor in the genesis of disease has been investigated by many workers. Most of their experiments have involved either the administration of an antigen to which the animal has previously been made sensitive or the administration of antibodies specific for certain tissues of the animal. In regard to this second group it has been hypothesized that the animal's own tissue acts as an antigen, reacting with the artificially provided antibody.

### HISTORY

Lindemann,<sup>1</sup> in 1900, while investigating the effect of various chemical and biologic poisons on the kidney, produced an antiserum specific for rabbit kidney by immunizing guinea pigs against the renal substance of rabbits. By administration of this specific antirenal serum to rabbits, he succeeded in producing severe tubular degeneration and necrosis. The glomeruli showed no changes except for small amounts of albumin in an occasional capsular space.

This work was confirmed by Pearce<sup>2</sup> in 1903. However, he showed that some of the effects observed previously were not due to antibodies specific for renal tissue but were due to antibodies specific for serum and red blood cells. To remove this variable factor, he introduced the procedure of perfusing the organs to be used as antigen with saline solution before they were removed and ground up.

In 1933 Masugi<sup>3</sup> succeeded in producing glomerulonephritis in rabbits by the use of nephrotoxic serum. In his original experiments rabbits were given repeated injections of a suspension of rat kidney, and their serum was subsequently injected into rats. Later he immunized ducks against rabbit kidney and then administered this antirenal serum to rabbits. On examination of the kidneys of these animals, he found proliferation of the glomerular endothelium, edema of the capillary walls, fibrinoid masses and hyalinization within the glomerular tuft, capsular exudate consisting of albumin, red blood cells and desquamated epithelium, adhesions between tuft and capsule, crescent formation and interstitial fibrosis with cellular infiltration.

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From the Department of Pathology, Northwestern University Medical School.

1 Lindemann, W. *Ann Inst Pasteur* **14** 49, 1900.

2 Pearce, R. M. *Univ Pennsylvania M Bull* **16** 217, 1904.

3 Masugi, M. *Beitr z path Anat u z allg Path* **92** 429, 1934.



The work of Masugi was confirmed and extended by Smadel,<sup>4</sup> Smadel and Farr,<sup>5</sup> and Swift and Smadel.<sup>6</sup> These authors reviewed the literature on renal lesions produced by the use of specific antirenal serum and reported that glomerulonephritis had been produced in rats by the use of such serum. They showed that by an adjustment of the dose of serum the chronic stage of the disease could be reproduced. Their conclusions were supported by clinical, functional and pathologic studies of the test animals. The authors differentiated between lesions caused by specific antirenal serum and those caused by serum containing antibodies specific for serum and blood cells, and emphasized the importance of removing all blood by perfusion from the organ to be used as antigen, as first advocated by Pearce. Recently, Seegal and Loeb<sup>7</sup> have shown that glomerulonephritis can be produced in rats by the injection of specific antiprenal serum. In addition, these authors showed that the "in vitro" titration of antiserum does not necessarily correspond to its activity in vivo.

In addition to administering specific antirenal serum, Masugi<sup>8</sup> also administered specific antihepatic serum to rats. Rabbits were immunized with a suspension of rat liver to provide the specific antiserum. He removed antibodies specific for the red blood cells and the serum of rats by mixing the rabbit serum with rat red blood cells and serum. This, he believed, would remove any antibodies to red blood cells or to serum by absorption. Subsequent serologic tests showed the precipitin titer of the mixed serums to be 0 for rat red blood cells, 80 for rat serum and 320 for rat liver. Twenty-four to forty-eight hours after injection of the mixed serum, autopsy showed hyaline changes, fatty metamorphosis and necrosis of liver cells, particularly in the peripheral and intermediate zones of the hepatic lobules. There was also cellular infiltration in which neutrophilic granulocytes, lymphocytes and many eosinophilic granulocytes participated.

In an attempt to produce cardiac lesions similar to those found in rheumatic fever, Bauer<sup>9</sup> prepared serum specific for rat heart by administering a suspension of rat heart to rabbits in successively larger doses. The rabbit serum showed precipitins specific for rat heart in a dilution of 1:4,000. There were also variable smaller amounts of antibody to rat blood serum as well. Rats receiving large doses (0.3 to 2 cc) of this antiserum into the femoral vein died within a few minutes to one-half hour with the clinical picture of anaphylactic shock. Autopsy

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4 Smadel, J. E. *J. Exper. Med.* **64**: 921, 1936, **65**: 541, 1937.

5 Smadel, J. E., and Farr, L. E. *J. Exper. Med.* **65**: 527, 1937.

6 Swift, H. F., and Smadel, J. E. *J. Exper. Med.* **65**: 557, 1937.

7 Seegal, B. C., and Loeb, E. N. *J. Exper. Med.* **84**: 211, 1946.

8 Masugi, M. *Beitr. z. path. Anat. u. z. allg. Path.* **91**: 82, 1933.

9 Bauer, F. C. *Arch. Path.* **42**: 222, 1946.

showed severe hyperemia, edema and hemorrhage of the lungs. Other organs showed only slight hyperemia. Smaller doses over a period of one to three weeks caused lesions in the lungs, which consisted of thickening of the alveolar walls with fibrinoid material and connective tissue cells. Rats receiving intraperitoneal injections showed marked fibrosis of the parietal and visceral peritoneum. The authors pointed out that the serum contained specific antibodies for all the tissues found in the heart, that is, cardiac muscle, connective tissue, endothelium and blood serum, and therefore the first of these tissues encountered in the recipient's body would react with the antibody and fix it. This was thought to explain the marked reactions of the lungs when the serum was injected intravenously and the peritonitis when it was injected intraperitoneally.<sup>10</sup> There were no significant findings in the hearts.

In consideration of these attempts to produce lesions in an organ by the administration of antiserum specific for the tissue of that organ, the question arose whether or not lesions might be produced in the liver by the administration of antihepatic serum. Masugi reported that relatively large doses of antihepatic serum produced microscopic lesions in the liver within twenty-four to forty-eight hours. The present study was undertaken to determine the effect of smaller doses of antihepatic serum administered over a period of months, as well as the effect of large doses administered for a few days.

#### MATERIAL AND METHODS

*Preparation of Specific Anti-Rat-Liver Serum*—White rats were killed with ether, and the livers were removed and perfused with isotonic solution of sodium chloride until the return fluid was clear. They were then chopped into small pieces and washed in continuously running tap water for twelve to eighteen hours. Then the pieces were placed in a mortar and pestle and ground until a smooth paste was prepared. It was found that sand was not necessary for grinding. The paste was diluted with isotonic solution of sodium chloride to make a 10 per cent suspension and merthiolate® was added to make a 1:5,000 concentration. After twenty-four hours this antigen suspension showed no growth on dextrose agar.

This liver suspension was administered intraperitoneally to 4 rabbits at three day intervals. The dose schedule is recorded in the table. No untoward reactions occurred. One week after the last injection was given, 10 cc of blood was taken from the ear vein of each rabbit to determine the antibody titer. This was done by the precipitin test recommended by Zinsser and Bayne-Jones.<sup>11</sup> Precipitation occurred in the serums of 3 rabbits in a dilution of 1:2,000 after one hour at room temperature and in that of the fourth rabbit in a dilution of 1:500. This rabbit was not used. Precipitation also occurred with rat serum (1:1,000 after one hour) and rat kidney (1:100 after two hours). There was no precipitation with rat heart

<sup>10</sup> Stenn, F. Arch. Path. 26:244, 1938.

<sup>11</sup> Zinsser, H., and Bayne-Jones, S. A Textbook of Bacteriology, New York, D. Appleton-Century Company, Inc., 1939, p. 932.

The 3 rabbits showing high titers were bled from the ear vein, about 50 cc of blood being taken from each. The serum was separated from the blood and pooled, and merthiolate® was added to make a 1:5,000 dilution. After twenty-four hours there was no growth on dextrose agar.

*Administration of Anti-Rat-Liver Serum to White Rats*—The animals were divided into two groups. Group A received large doses for a short period (2 cc every other day for two days, five days and two weeks), and group B received small doses for a long period (0.3 cc every other day for three months). Each group was divided into three series. Series P received the liver antiserum, series N received normal rabbit serum, series A received a 5 per cent solution of egg albumin. All injections were intraperitoneal. The doses were the same for experimental and control animals in each group. Three other rats were maintained on the same diet as the experimental animals as food controls. All animals were fed a commercial dog chow<sup>12</sup> and allowed as much water as they would drink. Their weights varied from 166 to 304 Gm. They were from a mixed laboratory strain, and the series included both males and females.

*Schedule for Administration of Rat Liver Antigen to Rabbits*

| Days | Cc of 10%<br>Rat Liver Antigen |
|------|--------------------------------|
| 1    | 1                              |
| 4    | 2                              |
| 7    | 3                              |
| 10   | 4                              |
| 13   | 6                              |
| 16   | 9                              |

The rats were killed with ether, and the autopsy material was fixed in buffered 4 per cent formaldehyde solution, Zenker's solution and 70 per cent alcohol. Sections were stained by the hematoxylin-eosin method, Mallory's technic for connective tissue, Mallory's phloxine technic, the silver reticulum method, Best's carmine technic for glycogen and with periodic acid.

# RESULTS

Within a few seconds after injection of the anti-rat-liver serum, the animals acted as though in extreme pain. Their heads drooped, their eyelids fell and finally the rats rolled on their sides and backs. They were quite restless and changed positions frequently. All rats seemed to recover after about five minutes. The severity of the reaction decreased with each injection, until, after about eight or ten injections, the rats no longer exhibited such signs following the administration of this serum. Those receiving albumin showed no reaction.

At autopsy there were no abnormal gross findings in any rats. The peritoneum was inspected for signs of peritonitis, but there were none. All organs were grossly normal. Of the 6 rats in group A, series P (large doses of antihepatic serum for short periods), the 2 animals that had been killed forty-eight hours after a single dose of 2 cc of the liver antiserum and the 2 that had been killed after four days of injections every other day showed scattered small granulomatous lesions, consisting of areas of necrosis of liver cells with infiltration of mononuclear cells, giant cells and a few lymphocytes and eosinophilic granulocytes. In addition there

<sup>12</sup> Wayne Dog Blox® food pellets

were occasional single giant cells scattered throughout the hepatic parenchyma. An occasional liver cell was swollen and filled with hyaline material, and its nucleus was fragmented. The kidneys, spleen, intestine and lungs were all histologically normal. The 2 animals in this group that received an injection every other day for two weeks did not show any lesions. The liver and other organs were histologically normal. The control animals (those that received normal serum or egg albumin and the food controls) did not have any histologic lesions in the liver or other organs. No histologic lesions were observed in the liver, the kidney or other organs in either the experimental or the control animals of group B. Best's carmine stain showed a slightly decreased amount of glycogen in the livers of all the animals, but there was no difference in glycogen content between the experimental and the food control animals.

#### COMMENT AND SUMMARY

In these experiments an anti-rat-liver serum was administered intraperitoneally to white rats for periods of two days, four days, two weeks and three months. The serum was prepared by injecting a suspension of ground perfused and washed rat livers into rabbits, and it had an anti-rat-liver titer of 1:2,000.

Scattered small granulomatous areas of focal necrosis and cellular infiltration were observed in the livers of the rats that had received one 2 cc dose, with autopsy forty-eight hours later, and in the rats that had received two 2 cc doses over four days, with autopsy forty-eight hours after the last dose. The rats that had received 2 cc of liver anti-serum every other day for two weeks and those that had received 0.3 cc every other day for three months, as well as all control animals to which normal rabbit serum or 5 per cent egg albumin had been administered, suffered no lesions in the livers, kidneys, lungs, peritoneum or other organs that could be detected with the naked eye or by ordinary histologic examination.

It is well to note here that Seegal and Loeb<sup>7</sup> have shown that the toxicity of an antiserum is not always directly proportional to its specific antibody titer.

The areas of focal necrosis occurring in the liver after only one or two injections of antihepatic serum is in agreement with the works of Masugi.<sup>8</sup> However, after the administration of this serum had been continued for weeks or months, it did not cause any lesions in the liver or other organs. One explanation for this observation may be the hypothesis that the liver was damaged by the initial dose of antiserum but quickly recovered and developed resistance or tolerance to subsequent doses.

# SIGNIFICANCE OF DUCTAL SCLEROSIS IN PAGET'S DISEASE

## Regression of Intraductal Carcinoma

RUDOLPH MARX, M D

LOS ANGELES

THAT carcinoma of the mammary ducts has been associated with Paget's disease of the nipple in most, if not all, of the cases studied is generally recognized. Haagensen<sup>1</sup> stated, "In Paget's erosion of the nipple meticulous microscopic search of the breast will always reveal somewhere in it a primary carcinoma whose cells have grown along the ducts to reach the surface of the nipple." However, there are instances in which a ductal carcinoma has not been found despite typical changes of Paget's disease in the nipple<sup>2</sup>, in some of the patients carcinoma was discovered subsequently in the regional nodes or elsewhere, indicating that it must have been present in the first place. In such cases it has generally been assumed that the carcinoma of the ducts must have been overlooked. Study of a recently observed case of Paget's disease of the nipple, in which ductal carcinoma was found only with difficulty and in which evidence of considerable regressive change was noted, has suggested another explanation of this puzzling phenomenon. Analysis of this case provides a clue to an understanding of those instances in which carcinoma of the ducts is apparently not present.

A 54 year old white woman had noted a small red area on the left nipple for seven weeks. This area burned and itched and did not respond to local applications. The nipple presented a small granular eroded area, biopsy resulted in a diagnosis of Paget's disease of the nipple (carcinoma simplex of the nipple). Mastectomy and axillary dissection were done.

Examination of the specimen (by Dr. Nathan B. Friedman) showed that the breast measured 16 cm. in greatest diameter and that the covering ellipse of skin was 15 by 6 cm. and contained an everted nipple, the surface of which was roughened and eroded. Microscopically, the skin of the nipple had isolated Paget's cells scattered about in the epidermis and occasionally larger aggregations of similar elements, which formed nests bulging into the underlying dermis similar to those seen in junctional nevi. An occasional cleavage plane had formed just above the basal layers of the epithelium. There was a moderate degree of inflammatory cellular infiltration along the dermoepidermal border.

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From Cedars of Lebanon Hospital

1 Haagensen, C. D. J. A. M. A. **138** 195, 1948

2 Geschickter, C. F. Diseases of the Breast, Philadelphia, J. B. Lippincott Company, 1945

Sections through the main ducts were not remarkable, nor were sections from many other portions of the breast. In view of the absence of microscopic changes in the mammary tissue itself, the entire gross specimen was subjected to meticulous



Fig 1—Microscopic view of nipple. The epidermis shows many swollen and bizarre Paget cells.

Fig 2—Sclerosis and calcification of obliterated ducts.

Fig 3—Intraductal carcinoma with periductal sclerosis.

reevaluation. This resulted in the disclosure of a small focus, slightly scarred, about 1 cm. in diameter, located about 5 cm. from the nipple. Tiny, gritty granules

were embedded in this focus. Microscopically, the scarred area revealed many masses of necrotic and calcified material, which was embedded in and surrounded by lamellated collagen suggesting preexisting tubular structures. Giant cells and macrophages were present next to some of the concretions. In other sections, where there was frank intraductal carcinoma, viable neoplastic tissue could be recognized in the lumens of several ducts in close juxtaposition to the necrotic and calcifying debris.

#### COMMENT

Cheatle and Cutler<sup>3</sup> described similar findings in Paget's disease and emphasized the hyperplasia of the subepithelial connective tissue of the ducts with considerable proliferation of elastic fibers. They interpreted this as possible evidence of regression of preexisting neoplasm in some areas. It is not possible on the basis of the present case to state whether complete regression of the ductal carcinoma can occur, although this is a possibility which should be looked for in the study of other cases. If such regression can be complete, the scarred ducts might be ignored during the search for carcinoma of the ducts, since it would not be realized that this scarring represented previous carcinoma. Such a combination of observations might account for cases in which no carcinoma of ducts has been found and even for those in which subsequent carcinomatous involvement of lymph nodes or a scar of the skin was disclosed.

#### SUMMARY

A case of Paget's disease of the nipple in which there was an underlying ductal carcinoma of the breast is described. The carcinoma was found only after the gross specimen had been scrutinized minutely. There was evidence of considerable regression of the neoplastic process with resultant sclerosis and calcification of the involved ducts. The finding of sclerosis of ducts in Paget's disease should be taken as possible evidence of preexistent carcinoma. This observation might explain those cases of Paget's disease in which carcinoma of the ducts has not been found.

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3 Cheatle, G. L., and Cutler, M. *Tumors of the Breast*, Philadelphia, J. B. Lippincott Company, 1931.

# Laboratory Methods and Technical Notes

## TISSUE CULTURE STAINING "IN SITU"

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AND

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PHILADELPHIA

SINCE 1930 when one of us (M E S) with Dr Lawrence W Smith<sup>1</sup> developed a simplified method of tissue culture, attempts to fix and stain the latter "in situ" have met with little success. Removing the clot from the large cover slip after fixation and subsequent embedding produced too much distortion for the study of cytoplasmic inclusions and nuclear detail. Our main difficulty was shrinkage and turbidity of clot with resultant poor clearing. Since the advent of Earle's<sup>2</sup> method both these obstacles have been overcome. If the stains we use are different from those of Dr Earle it is not that we find his unsatisfactory but that for our particular purpose, i e, a study of human tumors, a more contrasting stain is desirable. The underlying principle of avoiding shrinkage by using graded mixtures of alcohol is identical. Our method is a combination of Earle's fixation and dehydration procedures and Masson's trichrome stain as modified by Pollak.<sup>3</sup>

### METHOD

The cover slips used in the present method are 62 by 62 by 1 mm, and the diameter of the rings is 28 mm. Five to six or more fragments of tissue are implanted in this ring and grown in 6 drops of medium. This medium varies in quality according to the cultures, but the total quantity always remains the same. The thickness of the clot is such that cellular detail is perceptible in different depths of the medium.

For the detailed preparation of solutions we refer to the original articles. All solutions, including formaldehyde and potassium dichromate solutions, must be filtered in order that cultures may be kept free from dirt particles, which adhere to the clot and cannot be washed off. City water (Philadelphia's) must be passed through ordinary filter paper (Fisher Scientific Company no 9-795 semicrimped rapid). Filtration of solutions is exceedingly important and cannot be over-emphasized.

Ten parts of 37 per cent formaldehyde solution are added to 100 parts of 3 per cent potassium dichromate solution immediately before using and filtered. The

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From the Agnes Barr Chase Cancer Research Foundation Temple University Medical School and Hospital

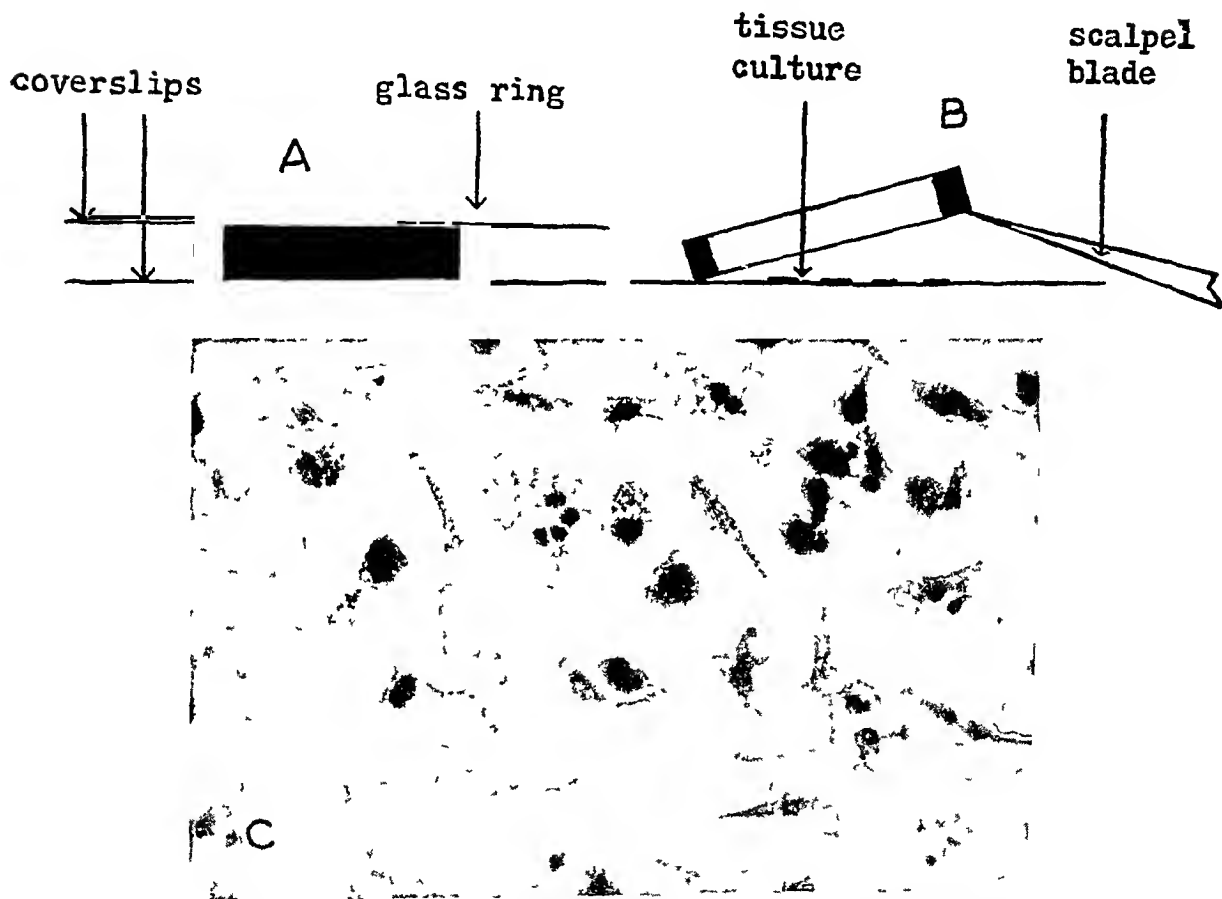
1 Sano, M E, and Smith, L W. Proc Soc Exper Biol & Med. **28** 282, 1930

2 Earle, W R. J Nat Cancer Inst **8** 83, 1947

3 Pollak, O J. Arch Path **37** 294, 1944



solution is warmed to 38 C in the incubator in a closed staining dish. The tissue culture to be stained is not removed from its oven until the fixing solution has attained the right temperature. To remove the top cover slip and ring from the tissue culture chamber a warmed Bard-Parker scalpel is passed over the top cover slip to melt the paraffin-petrolatum U S P seal. The cover slip then slides off easily. The ring is pried loose with the warm scalpel from the bottom cover slip on which the culture has grown. Any accumulated liquid is allowed to drain off the culture. Gently immerse the cover slip in the warmed formaldehyde-dichromate mixture, making certain the cover slip stands on end and does not lie flat on the bottom of the dish. Place in the incubator at 38 C for fifteen minutes. Refresh the



A, tissue culture chamber. In B the top cover slip has been removed. The ring is pried loose from the tissue culture cover slip. C, tissue culture of centrifuged cells from a tuberculous pleural effusion. Note giant cells, lymphocytes and fibroblasts.

fixative. Allow to stand overnight in freshly prepared fixative at room temperature. Change the fixative next day if the solution darkens. After twenty-four hours remove the cover slip from the fixing solution, avoiding the use of metals, and rinse in distilled water. Immediately place the cover slip in filtered 3 per cent potassium dichromate solution (without formaldehyde solution) for twenty-four hours. The culture can remain in this solution for seventy-two hours if necessary without the appearance of the cells being changed. The cover slip is now washed for twenty-four hours in filtered running tap water, care being taken that the cover

slip stands on end After twenty-four hours the culture is placed in alcohol (1 part), glycerin (1 part), distilled water (2 parts) mixture Best results are obtained when it is left in this mixture overnight Again, if it is left seventy-two hours in this solution, no deleterious effects are noted

The fixed tissue culture is now ready for staining It is placed in Harris' hematoxylin for two minutes (as in all staining procedures the period may vary according to the individual batch of stain) Drain the staining solution off and wash for twenty minutes in filtered running tap water Stain in Pollak's <sup>3</sup> trichrome solution for ten to fifteen minutes Differentiate shortly in acidified distilled water (0.2 per cent acetic acid)

Dehydrate through 35, 50, 70, 80, 95 per cent alcohol and then absolute alcohol, three minutes in each solution Clear in Earle's series of six solutions, absolute alcohol, acetone and toluene mixtures, five minutes in each solution Mount in clarite<sup>®</sup> dissolved in toluene of a 3 by 2 inch (7.6 by 5 cm) slide of standard thickness

#### COMMENT

Like tissue culture itself, the method is not difficult to master once the necessary steps have been well established The tissue culture method referred to <sup>1</sup> permits the study of the cells under high power and under oil immersion if desired Later, the modified staining method described in the foregoing section of this article permits the study of these very same cells after fixation The method has an advantage over the regular hanging drop method in permitting simultaneous studies of numerous fragments It is inexpensive and simple, and it allows for endless variation of experiments

## Books Received

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THE RENAL ORIGIN OF HYPERTENSION By Harry Goldblatt, M D, C M, director, Institute for Medical Research, Cedars of Lebanon Hospital, and professor of pathology, University of Southern California, Los Angeles Publication no 14, American Lecture Series A Monograph in American Lectures in Pathology Pp 126 Price \$2.75 Springfield, Ill Charles C Thomas, Publisher, 1948

This is an excellent summary of present day knowledge concerning the renal origin of hypertension. Written by one of the leading specialists, every chapter bears the imprint of intimate familiarity with the subject, gained in many years of research.

Although the work of others is also considered, the volume is mainly based on the author's investigations. This would be a disadvantage in the case of most monographs, but Goldblatt has done so much to increase knowledge in the field of renal hypertension that this manner of presentation actually adds to the value of the book.

It is perhaps somewhat inconvenient that the bibliographic list includes only six entries. However, as the author suggests, these key references will help the reader to find additional pertinent original publications. This system may be somewhat inconvenient, but as the book was primarily written for those not specializing in research on hypertension, it will not be felt as a great drawback.

It would be difficult to give more information concerning the well established aspects of this vast subject in the space of 126 pages. The volume is attractively bound, illustrations, composition and paper all reach that high degree of excellence to which readers have been accustomed in Charles C Thomas books. The volume is highly recommended to all those interested in hypertension.

## EFFECTS OF VARIOUS DEGREES OF PROTEIN DEPLETION ON HISTOLOGIC AND CHEMICAL STRUCTURE OF RAT LIVER

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AND

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CHICAGO

**P**ROLONGED feeding of low protein, but otherwise adequate, diets results in significant alterations of the morphologic aspects and chemistry of the rat's liver. Although several reports describe one stage in the changes occurring in the liver during inanition or protein deprivation, few serial studies on the effects of protein depletion have appeared. In some of these studies the period the animals were fed the low protein ration was apparently too short to reveal major changes. Chemical analyses were usually performed on one group of livers, while histologic descriptions were made from other experiments, often by different investigators. Therefore, it seemed of value to study the simultaneous chemical and histologic transformations taking place in the rat liver during protein depletion.

### MATERIAL AND METHOD

Young adult white male Sprague-Dawley<sup>3</sup> rats were used. Fed Wayne dog chow ad libitum until they weighed 200 to 220 Gm, these rats were then offered our standard low protein (3E) diet<sup>1</sup>. This ration contained 4 per cent fat (corn oil), so carbohydrate provided most of the calories. The diet also contained yeast, liver concentrate and corn starch, which have a moderate to high choline content,<sup>2</sup> and was supplemented with 10 mg of choline chloride per gram of diet.

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The work has been aided by the Douglas Smith Foundation for Medical Research of the University of Chicago.

The research which this paper reports was undertaken in cooperation with the Navy Department Office of Naval Research. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or the indorsement of the War Department.

1 Wissler, R W, Woolridge, R L, Steffee, C H, and Cannon, P R. *J Immunol* **52** 267, 1946.

2 Fletcher, J P, Best, C H, and Solandt, O M. *Biochem J* **29** 2278 1935.

The total amount of choline thus provided was about 14 mg per gram of ration. The animals, 7 to a cage, received 15 Gm per rat per day. After 30 days the amount of food was increased to 20 Gm to insure adequate dietary consumption on the part of competing animals and to produce more uniform depletion. This regimen approximated *ad libitum* feeding and guaranteed the intake of sufficient amounts of vitamins, minerals, accessory factors and calories needed for growth. Therefore, the lack of protein was the only known primary deficiency. Groups of 5 rats of comparable initial weight were selected from larger lots which had eaten the 3E ration for 0, 17, 32, 43, 56 and 100 days. These animals, kept in individual cages, were fed the same diet for a final 11 day period. Rats on the ration for a total of less than 30 days received 15 Gm per day, while those fed for over 30 days were given 20 Gm. During these 11 days all animals ate 94 to 99 per cent of the diet offered, except for the last two groups which averaged 85 and 72 per cent, respectively, but still maintained adequate caloric intakes. A control group of 5 animals of similar initial body weights was selected from rats which were offered only dog chow and were never individualized. At the end of the depletion period animals had eaten the protein-deficient diet for 0, 11, 28, 43, 54, 67 and 111 days. All animals were killed on the same day under light ether anesthesia after a 12 to 16 hour fast with free access to water.

After withdrawal of about 40 per cent of the total blood volume, the liver was removed and weighed. A carcass weight without the liver but with the emptied gastrointestinal tract was obtained. Two samples of each liver were analyzed for the quantities of water, fat, protein residue and water-soluble fraction by a gravimetric method. Details of this procedure will appear in a later paper. A somewhat similar gravimetric analysis was employed by Addis and co-workers.<sup>4</sup> In our method the water-soluble fraction represents the material removed by water at 70 C after the tissue has been coagulated by heat and extracted with organic solvents (alcohol and ether). The "protein residue" determined after completion of the extractions contains 90 to 95 per cent protein, calculated as NX6.25.

Analyses of carcasses were performed by a similar procedure described elsewhere.<sup>5</sup> Other samples of each liver were fixed in 4 per cent formaldehyde in saline solution. Frozen sections were stained for fat with oil red-O and counter-stained with hematoxylin. Celloidin-embedded, hematoxylin and eosin preparations were made.

In another experiment a group of 4 rats which had eaten the 3E diet for 77 days was subjected to removal of approximately 30 per cent of liver tissue under ether anesthesia. Fat stain and hematoxylin and eosin sections were prepared from the resected specimens, but no chemical analyses were done. After a 14 day post-operative period, during which they received 15 Gm per rat per day of a 9 per cent lactalbumin-casein diet (diets D),<sup>5</sup> the animals were killed and analyzed in the manner described in the foregoing paragraph. Four other rats were treated similarly, but no partial hepatectomy was performed. Thus, these animals provided an evaluation of the state of the liver at 77 days and demonstrated the effects of protein repletion.

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3 Footnote deleted by the authors

4 Addis, T., Poo, L. J., Lew, W., and Yeun, D. W. *J Biol Chem* **113** 497, 1936

5 Benditt, E. P., Humphreys, E. M., Wissler, R. W., Steffee, C. H., Frazier, L. E., and Cannon, P. R. *J Lab & Clin Med* **33** 257, 1948

## OBSERVATIONS

The estimates based on the chemical analyses are presented in the table as averages for the animals in each group, with the standard errors. Figure 1 gives the percentage composition of the livers. Representative photomicrographs were taken at the same magnification, each showing a typical liver lobule, to permit comparison of lobules with respect to size.

The normal fasted rat liver contained few stainable fat droplets, mostly in the peripheral areas near the portal triads (fig 2 *A*). Each lobule was orderly, with distinct sinusoids, eosinophilic cytoplasm and regular nuclei (fig 2 *B*). The "protein storage" bodies of Berg<sup>6</sup> stood out as dark reddish granules. Weighing about 7 Gm, the organ contained approximately 70 per cent water, 20 per cent protein residue and 7 per cent fat.

After 11 days on low protein intake, a definite increase of small fat droplets appeared. This lipid material had a zonal distribution, mostly about the central vein of each lobule (fig 3 *A*). The lobule had decreased slightly in size, and the individual cells were smaller. Although the cytoplasm was a little more reticulated and paler, the protein bodies were still distinct (fig 3 *B*). A reduction of liver weight, due largely to a fall in water and protein residue content, accompanied this change. No alteration of estimated quantity of fat was noted, but a significant rise of the percentage occurred.

Twenty-eight days of the protein-deficient diet produced a more severe alteration. Droplets of fat contained in the hepatic cells occupied almost the entire lobule, and a few medium and large globules were evident in the midzonal and peripheral portions (fig 4 *A*). Closer approximation of central veins indicated decrease of lobule size. Almost normal cytoplasm with abundant eosinophilic granules was present in the cells adjacent to the central veins. Focal areas of atrophy of liver cells occurred in the midzonal and peripheral regions. Densely packed small dark nuclei gave evidence of decrease in individual cell size (fig 4 *B*). The sinusoids and the margins of cells were indistinct. This histologic change paralleled an increase of liver weight and fat content. Although every component but the water-soluble fraction increased in estimated quantity, the proportion of protein residue fell to about 18 per cent and that of water to 67 per cent, while that of fat rose to 12 per cent. Apparently, a change in the composition of the liver substance occurred in which fat accumulated at the expense of water and protein.

At 43 days the atrophy of liver cells involved the whole parenchyma. The cells were small, with compact nuclei, and the cytoplasm was uniformly pale, with less distinct "protein storage" particles. Lobules were smaller, with thin hepatic cords. The nuclei were regular, except for occasional large forms with more than two nucleoli (fig 5 *B*). In the peripheral areas medium and large droplets of stainable fat were scattered throughout liver cells with many fine globules, but the central regions tended to be relatively free of them (fig 5 *A*). The livers decreased in weight. Water, fat and protein residue quantities diminished, but little change occurred in the percentage composition, except for the drop in the fat component.

Between 43 and 111 days few additional histologic changes appeared. Sections of livers of rats depleted for 43, 54 and 67 days were not distinguishable. The livers became progressively smaller. The estimated quantities of water, fat, protein residue and water-soluble fraction decreased equally, with little variation in percentage composition.

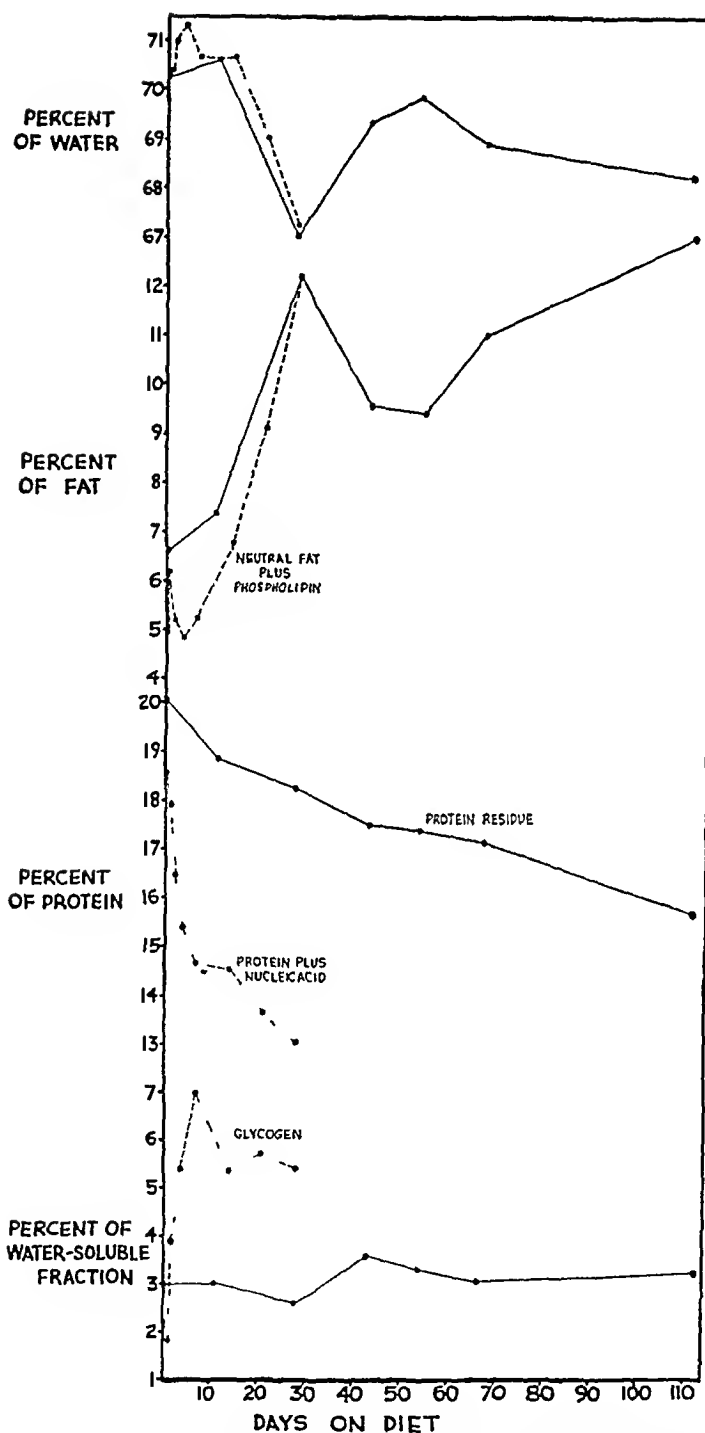


Fig 1—The average percentage composition of the liver at various stages of protein depletion. The broken line represents the data from Kosterlitz<sup>16</sup> as grams per hundred grams of liver.

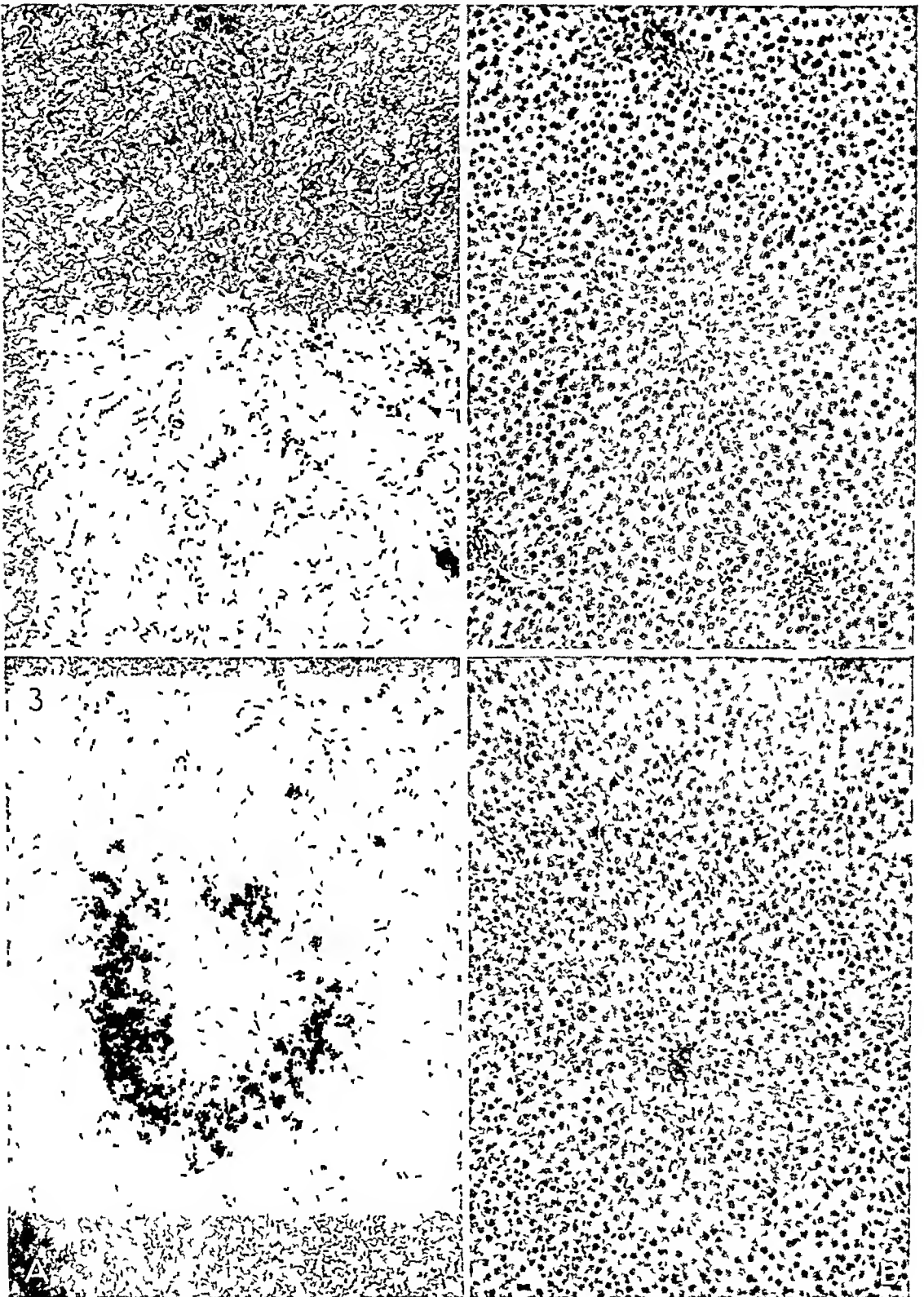


Fig 2—Normal fasted rat liver A, oil red-O and hematoxylin B, hematoxylin and eosin,  $\times 235$

Fig 3—Rat liver after 11 days of a low protein diet A, small droplets of fat accumulated near a central vein, oil red-O and hematoxylin B, slight decrease in lobule and cell size Hematoxylin and eosin,  $\times 235$



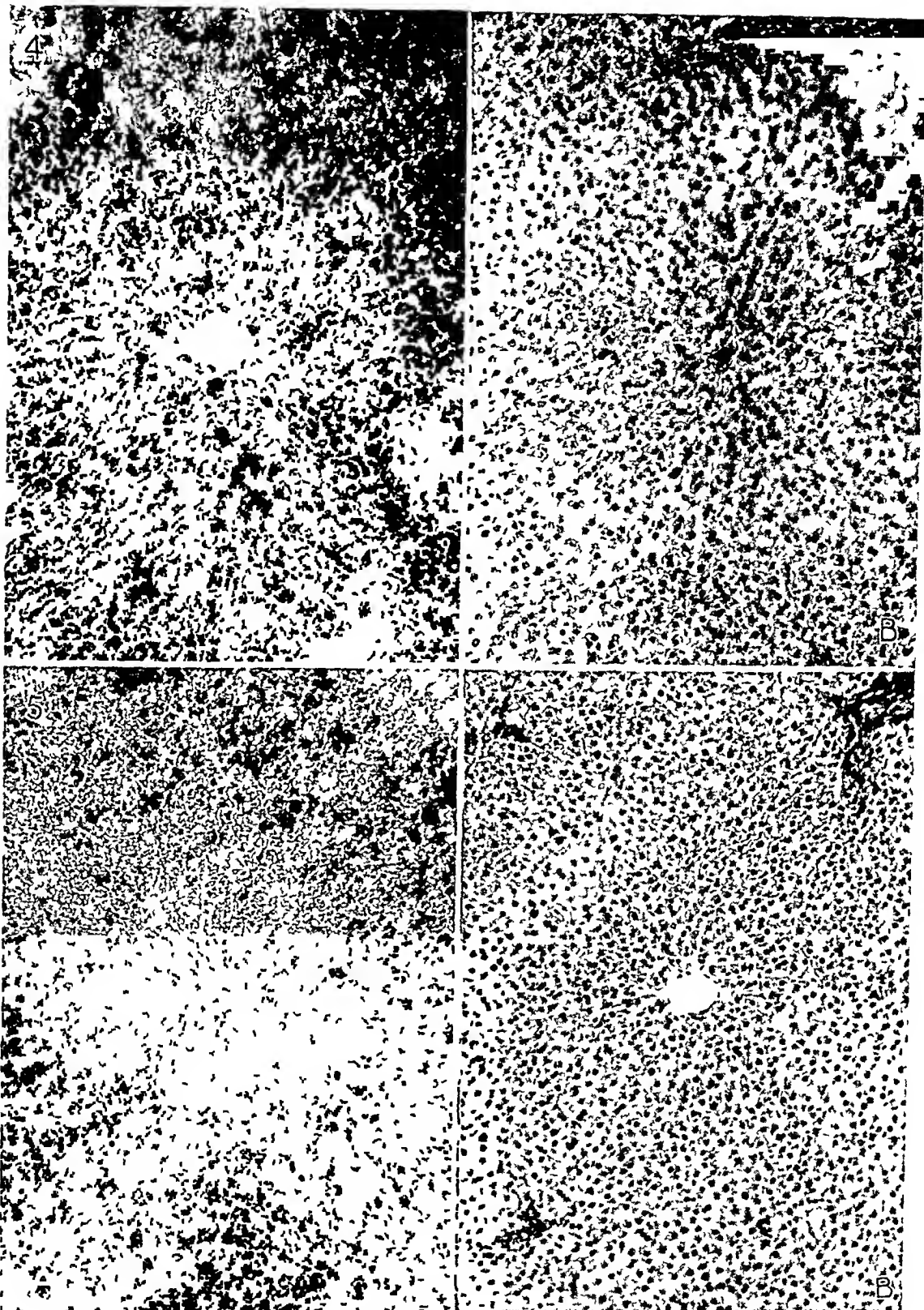


Fig 4—Rat liver after 28 days of low protein diet *A*, diffuse small droplet fatty change with larger globules in outer portions, oil red-O and hematoxylin *B*, focal atrophy of hepatic cells in peripheral areas, and peripheral cells with foamy cytoplasm, hematoxylin and eosin,  $\times 235$

Fig 5—Rat liver after 43 days of low protein diet *A*, peripheral small droplet fatty change with little in the central zone, oil red-O and hematoxylin *B*, small lobule with general atrophy, hematoxylin and eosin,  $\times 235$

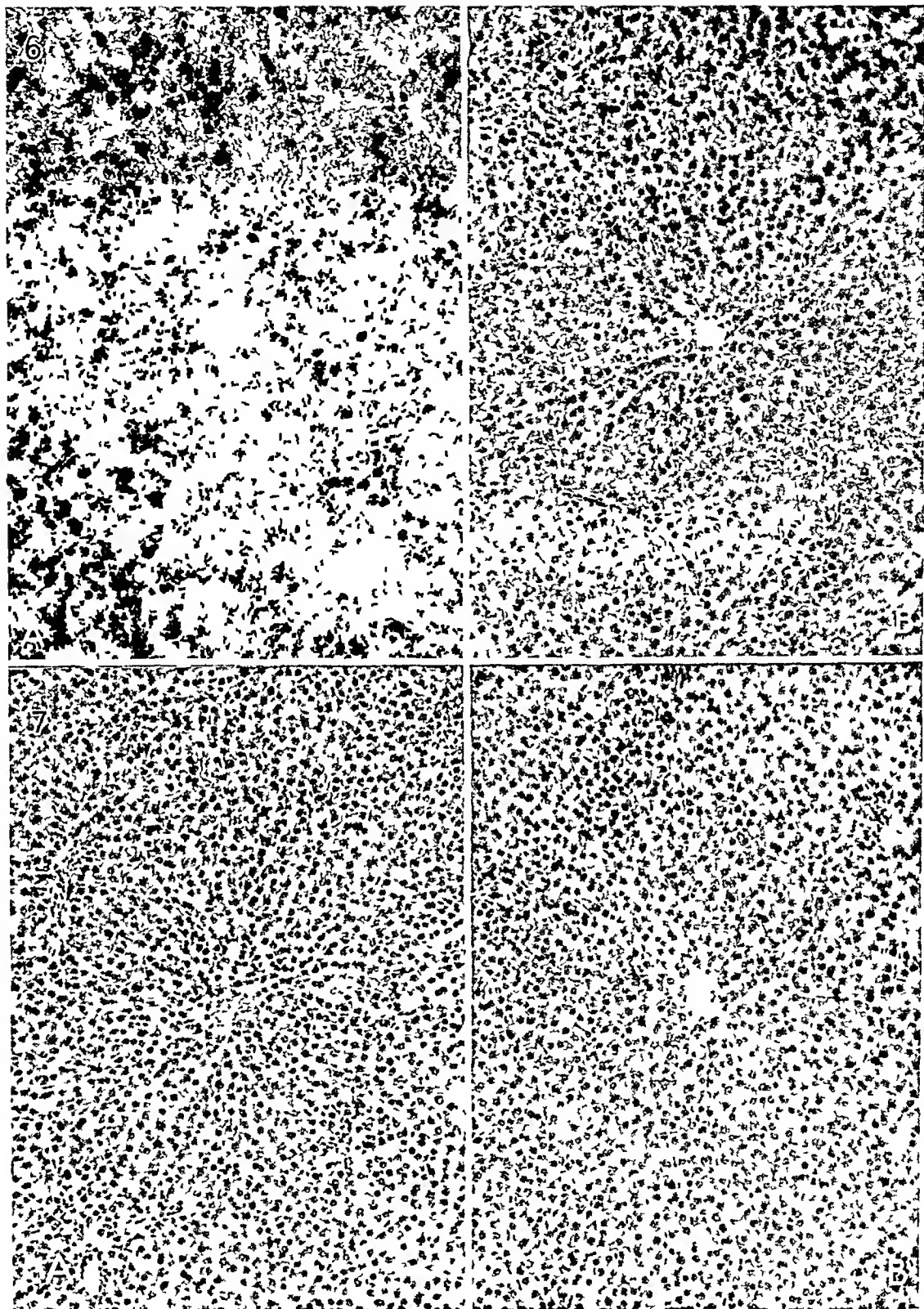


Fig 6—Rat liver after 111 days of low protein diet *A*, diffuse small droplet fatty change throughout lobule, oil red-O and hematoxylin *B*, small lobule with thin cells, hematoxylin and eosin,  $\times 235$

Fig 7—*A*, rat liver after 77 days of low protein diet Thin hepatic cords, narrow cells and slightly foamy cytoplasm Partial hepatectomy specimen *B*, liver of same rat after 14 days of 9 per cent protein diet Normal cells with few vacuoles Hematoxylin and eosin,  $\times 235$

After 111 days of protein depletion, a diffuse small droplet fatty change was again evident (fig 6A). The lobules were smaller than the normal, and the cords were narrow and close together. Individual cells consisted of a nucleus with a thin shell of faintly granular, pale, eosinophilic cytoplasm. The small dark nuclei were frequently replaced by large forms containing several nucleoli and clumps of chromatin. Some nuclei appeared to have undergone division (fig 6B). A decrease in estimated quantity and percentage of protein residue occurred between 67 and 111 days. However, the fat content rose, and its percentage value exceeded the 28 day value. The quantity of water changed insignificantly, but the liver was slightly larger than at 67 days.

That these progressive changes were reversible was illustrated by the sections and analyses of the animals subjected to partial hepatectomy (table). After 77 days of the low protein ration, the microscopic appearance of the liver resembled

*Chemical Estimations of Components of Livers and Carcasses of Animals  
Restricted to Low Protein Diets\**

| Animals | Days on<br>3E Low<br>Protein<br>Diet | Days on<br>9% Lact<br>albumin<br>Casein Diet | Liver<br>Weight,<br>Gm | Water,<br>Gm | Fat,<br>Gm   | Protein<br>Residue,<br>Gm | Water<br>Soluble<br>Fraction,<br>Gm | Carcass<br>Weight,<br>Gm | Carcass<br>Fat,<br>Gm |
|---------|--------------------------------------|--|------------------------|--------------|--------------|---------------------------|-------------------------------------|--------------------------|-----------------------|
| 5       | 0                                    | 0  | 6.67 ± 0.296           | 4.66 ± 0.206 | 0.44 ± 0.028 | 1.37 ± 0.062              | 0.20 ± 0.008                        | 178.6 ± 2.32             | 20.4 ± 1.81           |
| 5       | 11                                   | 0  | 5.67 ± 0.134           | 4.00 ± 0.107 | 0.42 ± 0.002 | 1.07 ± 0.040              | 0.17 ± 0.006                        | 153.6 ± 1.73             | 15.4 ± 1.43           |
| 5       | 28                                   | 0  | 6.84 ± 0.267           | 4.08 ± 0.164 | 0.84 ± 0.077 | 1.25 ± 0.067              | 0.18 ± 0.027                        | 149.2 ± 2.28             | 15.5 ± 0.92           |
| 5       | 43                                   | 0  | 5.22 ± 0.245           | 3.62 ± 0.161 | 0.50 ± 0.063 | 0.91 ± 0.045              | 0.19 ± 0.013                        | 151.6 ± 3.00             | 22.8 ± 1.08           |
| 5       | 54                                   | 0  | 4.83 ± 0.294           | 3.37 ± 0.180 | 0.46 ± 0.068 | 0.84 ± 0.042              | 0.16 ± 0.015                        | 143.2 ± 2.32             | 17.3 ± 1.14           |
| 5       | 67                                   | 0  | 4.28 ± 0.142           | 2.95 ± 0.084 | 0.47 ± 0.075 | 0.73 ± 0.020              | 0.13 ± 0.005                        | 132.0 ± 1.86             | 15.6 ± 1.49           |
| 4       | 111                                  | 0  | 4.49 ± 0.280           | 3.06 ± 0.170 | 0.58 ± 0.070 | 0.70 ± 0.041              | 0.14 ± 0.013                        | 114.7 ± 4.36             | 11.9 ± 1.43           |
| 4†      | 77                                   | 14   | 5.89 ± 0.170           | 4.16 ± 0.132 | 0.42 ± 0.020 | 1.18 ± 0.042              | 0.13 ± 0.008                        | 201.4 ± 3.52             | 29.5 ± 1.13           |
| 4       | 77                                   | 14   | 6.61 ± 0.228           | 4.65 ± 0.148 | 0.45 ± 0.022 | 1.38 ± 0.056              | 0.13 ± 0.018                        | 205.6 ± 4.15             | 28.6 ± 2.46           |

\* The values given are the means and standard errors for liver weight, carcass weight and the estimated amounts of the chemical constituents of the liver and the carcass. Standard errors were computed by

$$S.E. = \sqrt{\frac{\sum (x-m)^2}{n(n-1)}}$$

† These animals were subjected to partial hepatectomy after 77 days on the 3E diet.

the stage between 67 and 111 days (fig 7A). The diffuse small, and occasional medium, droplet fatty change tended to occupy the central and midzonal regions. After 14 days of repletion on the 9 per cent protein diet the prominent accumulation of fat had disappeared. Only a few isolated medium to large intracellular globules, surrounded by a ring of small droplets, were seen. The cords were thick with large rectangular cells containing a granular, eosinophilic cytoplasm with infrequent vacuoles (fig 7B). The sections were indistinguishable from those of normal rat livers. Since regeneration was not complete at the time of death, these livers were smaller than those of the animals not operated on. However, the percentage values of all components measured were almost identical with those of the control and the nondepleted rats.

This chronologic description of the changes that occurred in the rat liver during prolonged low protein feeding reveals several salient points. A definite fatty deposition occurs in which small droplets first appear near the central vein and later extend peripherally to involve the entire lobule. After 28 days this fatty change diminishes, with lipid disappearing first from those areas where it initially

was visible. The small fat droplets tend to reappear after moderate depletion of protein. The quantitative and percentage fat content parallels this histologic progression. An initial percentage increase of fat takes place in the first stage without a significant rise in the estimated quantity of lipid. When the entire lobule is affected by the fatty change, the fat content reaches a maximum chemically. A slight decrease of visible fat follows, accompanied by a decline of the percentage and the estimated quantity of lipids. With the late rise of the fat values the small droplets reappear. An interesting phenomenon is the apparent coalescence of the small droplets of the early fatty phase to form medium and large globules in the peripheral zones. Such fat globules are the last to disappear during the period of recovery produced by the repletion diet. Loss of protein residue is most marked during the first 11 days and is followed by a secondary increase at 28 days in this experiment. A steady decline of quantity and of percentage of protein residue accompanies the subsequent decrease of size of the whole liver lobule. The fact that individual liver cells become smaller during this process is apparently correlated with a loss of protein and a small loss of water. Though its estimated quantity decreases, the percentage of the water-soluble fraction, which includes glycogen, remains relatively stable during depletion of protein. All these manifestations of abnormal metabolism are brought to near normal levels by a short period on the 9 per cent protein diet.

Of as great importance as the changes of estimated quantities and of percentages of the liver components are the alterations which take place in the relations of these constituents, one to another. After 11 days of protein depletion, a progressive increase of water binding by the dried fat-free solids occurs. The maximum ratio of water to fat-free solids is attained at 111 days. This augmented water binding is associated with a decreased proportion of protein residue and a relative increase of the water-soluble fraction in the fat-free solids. Although less protein residue and water-soluble fraction are present, relatively more elements of the latter are available to hold water. Throughout the period of depletion the ratio of fat to protein residue rises steadily, reaching the highest value at 111 days. This increase parallels the microscopic evaluation of the quantity of stainable lipid. At 111 days the fall of protein residue quantity, associated with the largest percentage of fat, accounts in part for the maximum value of the ratio of fat to protein residue at this stage. After 14 days of the repletion diet, these proportions, as well as the quantitative and percentage values and the histologic changes, are returned to essentially normal levels.

#### COMMENT

Previous reports of the anatomic changes that occur in the livers of animals fed low protein diets have dealt mostly with the changes of size and the appearance and disappearance of "protein storage" particles. These granules were shown by Berg<sup>6</sup> to resemble closely the split products of protein microscopically. They disappeared on protein deprivation, and after bleeding, starvation, muscular activity and phlorhizin poisoning.<sup>7</sup> Carbohydrate and fat feeding failed to cause their reappearance, and only protein, either plant or animal,<sup>7</sup> brought about their formation. Even protein hydrolysates presumably con-

<sup>7</sup> Li, H. M. *Chinese J. Physiol.* **10** 7, 1936

taining only amino acids, led to restoration of these granules<sup>8</sup> Recent studies indicate that the storage of protein in the liver, first suggested by Pfluger,<sup>9</sup> does not depend on these particles but is, instead, a function of the entire cytoplasmic structure of the cell<sup>10</sup> Consistently, investigators observed that the liver cell was largest after ingestion of a high protein diet<sup>11</sup> Histologic evidence of fat deposition from protein deficiency received infrequent mention<sup>12</sup> Generally, the liver was described as showing under these conditions a decrease of cellular cytoplasm with disappearance of eosinophilic granules and occasional accumulations of fat

Investigators have studied the chemical changes resulting from starvation more than those of primary protein deficiency with adequate intake of other dietary essentials These chemical studies of hepatic protein depletion have revealed decreased amounts of protein, elevated water levels and usually unchanged fat contents Rapid loss of as much as 40 per cent of total liver protein occurred when rats were fasted for 7 days, while 20 per cent disappeared in 2 days<sup>13</sup> On low protein or protein-free rations the total nitrogen content of dog livers was found to decrease steadily, while the percentage remained nearly constant<sup>14</sup> Percentage water values were reported as elevated in these experiments, but no change occurred in the liver fat

Elman and co-workers<sup>15</sup> have described a correlation between the chemical and the histologic changes in dogs and rats fed a low and a nonprotein diet The small cells and narrow cords seen in the livers of animals fasted after 3 weeks of depletion resembled those observed in our experiment However, it is impossible to compare our results with theirs, since the animal species, the composition of the diets, the length of time of depletion and other conditions are not comparable

A recent publication by Kosterlitz<sup>16</sup> described the changes in composition and structure of the liver when different types and amounts of protein were fed female rats A 60 per cent casein diet led to livers and cells which were larger than those of rats fed the stock diet, although the protein percentage was slightly lower Low or poor protein rations (18 per cent gelatin and 8 per cent yeast, 8 per cent yeast) resulted in small livers with decreased percentage of protein, but the

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8 Berg, W, and Cahn-Bronner, C *Biochem Ztschr* **61** 434, 1914

9 Pfluger, E *Arch f d ges Physiol* **96** 1, 1903

10 Luck, J M *J Biol Chem* **115** 491, 1936

11 Noël, R *Arch d'anat micr* **19** 1, 1923

12 Bruning, H *Jahrb f Kinderh* **79** 305, 1914

13 Addis, T, Poo, L J, and Lew, W *J Biol Chem* **115** 111 and 117, 1936

14 Elman, R, and Heifetz, C J *J Exper Med* **73** 417, 1941

15 Elman, R, Smith, M G, and Sachar, L A *Gastroenterology* **1** 24, 1943

16 Kosterlitz, H W *J Physiol* **106** 194, 1947

cells were of normal size. When he fed an essentially protein-free diet, containing 10 per cent lard, for 28 days, he noted a marked decrease in liver weight and protein percentage during the first 22 to 46 hours. The protein continued to diminish, but the liver size remained relatively constant during the next 26 days. In the first 22 hours the percentage of fat (neutral lipid plus phospholipin) rose, but returned to normal by the end of 4 days, and then showed a continuous rise which became steeper during the last 14 days. When his values per hundred grams of liver are superimposed on the curves from our experiment (fig. 1), a striking similarity is observed in the percentages of water and fat. It is impossible to compare the glycogen and protein values directly, because of the differences in diets and methods.

The histologic changes described by Kosterlitz included loss of basophil granules. These changes resembled those observed in our lobular peripheries, and coincided with the decrease of protein, phospholipin and nucleic acid percentages, with a rise of glycogen and, in the later stages, also of lipid. Starving the rats for 48 hours after the protein-free regimen resulted in small, homogeneous cells with decreased basophil granules. These changes resembled those observed in our experiment, in which the animals were fasted for 12 to 16 hours. The alterations due to starvation were attributed mainly to variations of glycogen content. Kosterlitz' study clarified some of the changes occurring in the early stages of protein depletion, and our results confirm his findings.

Since the body is constantly destroying some protein, a deficient supply would be expected to result in a loss of tissue substance. Labile protein reserves are maintained in the liver, but during inanition or protein deficiency they are mobilized and utilized in the body's metabolism. All parts of the cell are affected by this depletion with the possible exception of the nucleus, for the nucleic acid concentrations are reported as increasing during low protein feeding<sup>17</sup>. With loss of tissue a decrease in water and, in our study, in glycogen (water-soluble fraction) would follow. Significant metabolic alterations probably occur during protein deprivation. That these changes do not necessarily take place simultaneously or at the same stage is indicated by the finding of variable and often divergent chemical and histologic results during the first 11 to 28 days.

Although others have not described close correlations between histologically visible fat and quantitative analyses,<sup>18</sup> a definite relationship appears in our study. The source of the fat which accumulates betw

17 Kosterlitz H. W. *Nature, London* **154** 257, 1944.

18 Rosenthal O. *Arch. Gén. de physiol.* **21**:573 1936.

11 and 28 days is uncertain Uher<sup>19</sup> found that in "fatty infiltration" the quantities of hepatic and subcutaneous fat parallel each other, and he concluded that the hepatic fat was transported from these reserves The data concerning carcass fat (table) suggests that some of the lipid material could have come from carcass depots Whether the change in storage fat is responsible for the increase in hepatic lipids, and why it appears in the manner and at the time it does, and why it later disappears remain for further investigation Although lipotropic factors must be considered, even the rats which were most depleted in our experiment (111 days) consumed approximately 20 mg of choline per day This quantity greatly exceeded the minimum daily amount needed to prevent fatty livers in normal rats<sup>20</sup> McHenry and Patterson<sup>21</sup> have emphasized that all fatty livers are not alike, nor are they alike in their response to lipotropic agents The fatty change produced by protein depletion does not depend on the absence of the lipotropic influence of choline When the basic diet with an adequate protein content (3 C)<sup>1</sup> is fed, rats grow and a normal liver is maintained

In human diseases which produce severe emaciation, such as tuberculosis and ulcerative colitis, fatty changes and atrophy of hepatic cells are often described Small droplets appear first in the periportal areas and may extend to occupy the entire lobules<sup>22</sup> The association of this pathologic state with conditions of poor nutrition suggests that a protein deficiency may determine its occurrence The apparent coalescence of small fat droplets into larger globules may mean that the changes of fatty degeneration and fatty infiltration tend toward a similar final histologic appearance Considering the resemblance between the microscopic changes in this experiment and those observed in human diseases, one concludes that protein deficiency may be prominent in the pathogenesis of the fatty liver of some "inanition diseases," as, for example, portal cirrhosis, celiac disease, esophageal carcinoma and chronic diarrheas This concept is supported by the fact that in these experimental animals, which are known to have ingested adequate amounts of accessory factors concerned with lipid metabolism, the only primary deficiency was protein deprivation

#### SUMMARY

An experiment made with young adult male rats illustrates the histologic and chemical changes which occur in the livers of animals restricted to a low protein diet for variable periods An initial fall in

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19 Uher, V *Ztschr f d ges exper Med* **96** 159, 1935

20 Griffith, W H, and Wade, N J *Proc Soc Exper Biol & Med* **41** 188 1939

21 McHenry, E W, and Patterson, J M *Physiol Rev* **24** 128, 1944

22 Jones, J M, and Peck, W M *Arch Int Med* **74** 371 1944



tissue mass parallels a decrease in lobule and cell size with the appearance of a small droplet fatty change. A secondary rise in liver weight is associated with a disproportionate increase in fat which is reflected in a diffuse fatty alteration. Subsequent depletion reduces both the quantities of water, fat, protein and water-soluble fraction and the visible cell size and fat content. After prolonged protein deprivation another increase in stainable and chemically detectable lipids occurs, while marked atrophy of cytoplasm and irregularity of nuclear arrangement appear.

Despite the severity of the histologic and chemical alterations, the entire process quickly undergoes complete reversal when a diet containing a high quality protein is fed.

The mechanisms which operate in the production of the hepatic changes of the cachectic states associated with some human diseases may be similar to those operating to produce the alterations observed in this experiment and may be induced by undernutrition, principally depletion of protein.



# PROLIFERATION OF THE EPITHELIUM OF INTRAHEPATIC BILE DUCTS IN PARABIOTIC RATS FOLLOWING OBSTRUCTION OF THE BILE DUCTS

ISOLDE T ZECKWER, M D  
PHILADELPHIA

MOORE, Hellman and Jacobius<sup>1</sup> have described experiments in which the common bile duct was ligated in one rat of a pair of parabiotic rats. Jaundice did not occur, as the normal twin excreted bile for both animals.

These experiments seemed to offer a technic which one might apply in studying the effect of a diversion of the flow of bile on the available estrogen in the body, and accordingly a number of rats were operated on. The experiments failed to serve that purpose, but the structural changes that occurred in the liver were striking and interesting in themselves and seemed to merit a brief recording. The histologic changes were concerned with much longer periods of obstruction than those in Moore's experiments. They illustrate the degree of proliferation of bile duct epithelium that can be maintained when a rat is kept alive by parabiosis for a much longer time than is possible in a single rat with obstruction of the common duct.

## METHODS

Parabiosis was effected by lateral incision of the abdominal wall of each rat and suture of peritoneum, muscle and skin, without open communication between peritoneal cavities, in litter mates of the same sex. After good union had been obtained, the common bile duct of one member of each pair was cut between ligatures. As these operations were done in the course of experiments on the interchange and production of hormones,<sup>2</sup> some of the rats had been castrated, some had been given injections of an estrogen and some had increased secretion of endogenous estrogen, but this altered endocrine status caused no important modifications in the liver that were recognized, except perhaps slight vacuolation of hepatic cells, probably due to deposition of glycogen, which is known to occur when an excess of estrogen exists.

Excluded from consideration were rats in which various accidents occurred, such as faults of surgical technic, hemorrhage, obstruction of blood vessels, infection, and early strangulation of the pedicle between the rats. It is not

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From the Department of Pathology, University of Pennsylvania School of Medicine.

This study was aided in part by a grant from the Faculty Research Committee of the University of Pennsylvania.

1 Moore, R A, Hellman, L M, and Jacobius, H. Arch Path **34** 196, 1942.

2 Zeckwer, I T. Endocrinology **38** 249, 1946.

easy to keep the pedicle between rats functioning for a long period. Experiments were successfully carried out on 5 pairs of male and 14 pairs of female rats for periods of 5 days to 114 days after ligation and severance of the common duct to cause biliary obstruction. Of these, 8 pairs were examined at late intervals, namely, 50, 56, 60, 100, 105, 107 and 114 days. At very early intervals no pairs were studied. Included in the series are rats in which the pedicle functioned well for a considerable length of time during obstruction, but in which eventually the pedicle became obstructed. In these rats the long-continued proliferative changes in the liver had superimposed on them the effects of failure to eliminate bile. Some rats were killed, others were studied after their spontaneous death.

Thirteen single rats with duct obstruction which survived longer than a week served for comparisons of the time of survival and the histologic changes. Many more single rats with duct obstruction died within a week.

### RESULTS

It was not possible to keep the single jaundiced rats alive longer than 39 days, whereas 8 pairs of parabiotic rats lived in good health for long periods. In the earlier stages of obstruction in the parabiotic rats, proliferation of bile ducts occurred in the portal triad. Soon extensive proliferation of bile duct epithelium extended into the interior of each lobule and replaced hepatic cells. Where hepatic cells still existed, intracellular canaliculi were distended but were empty or contained a slight amount of fleecy protein precipitate. In the midst of new ducts a few hepatic cells survived as individual scattered cells. The centers of lobules retained nearly normal structure.

In the late stages the bile duct epithelial cells were far more numerous than the hepatic cells. So few hepatic cells remained that it is doubtful if the function of the liver would have been adequate if the partner had not functioned for the obstructed rat. Where hepatic cells survived, many seemed to be regenerating, as evidenced by darkly stained, compact cytoplasm and nuclei that were unusually dark with chromatin. Occasionally minute areas of necrosis occurred. More commonly, however, hepatic cells near bile ducts showed only minor retrograde changes.

Grossly, the large ducts were greatly distended with clear, almost colorless fluid. In late stages the liver was pale, granular on the surface and very tough when sectioned. At 100 days after the ligation and severance of the common duct the liver was more than twice as large and twice as heavy as that of the normal partner.

In the single rats with obstruction of the common bile duct, intense hyperplasia of duct epithelium occurred, but the process was of shorter duration, as the injurious effects of retention of the bile were incompatible with life.

### COMMENT

Moore and his co-workers<sup>1</sup> emphasized the necrosis in the livers of most of the rats subjected to ligation of the common bile duct and also in the livers of 3 normal partners. A 36 day period was the longest period during which they studied 15 pairs of parabiotic rats and 15 single rats. In the present experiments the object was to study such rats at longer intervals. No necrosis was ever observed in the normal partner. In the partner with the common bile duct obstructed, individual hepatic cells were necrotic, but in the long time experiments

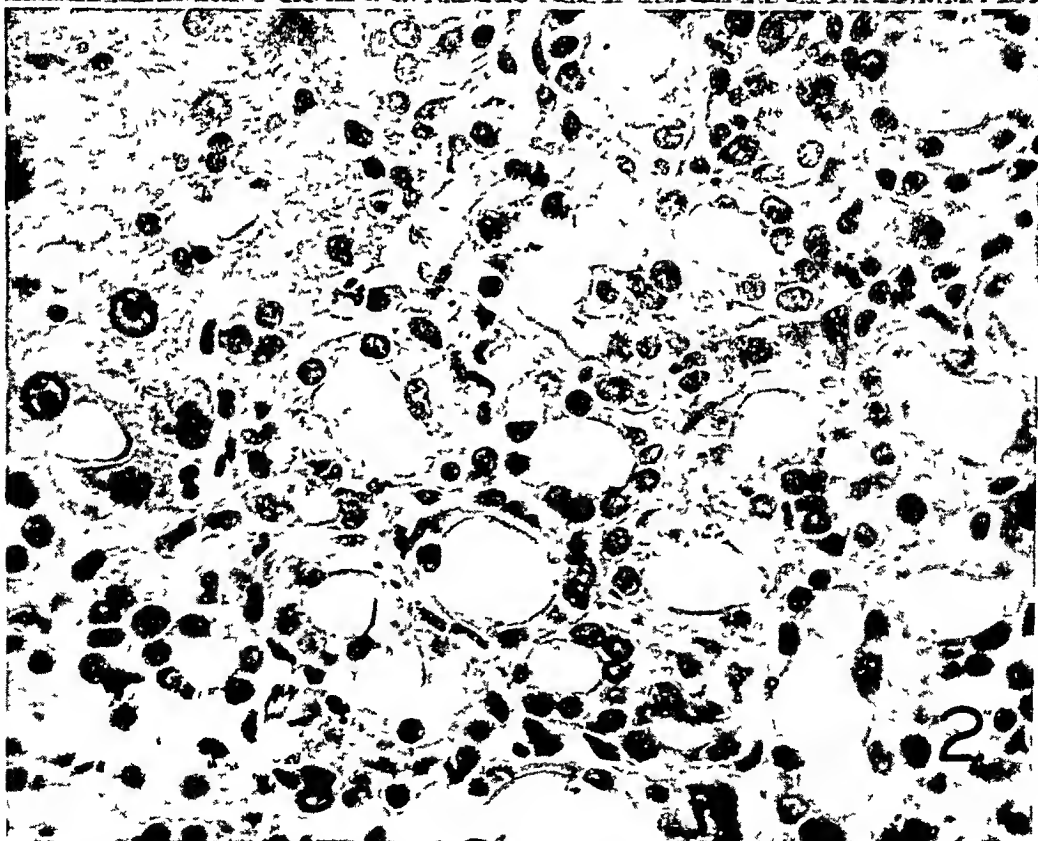


Fig 1—Liver ( $\times 122$ ) of a parabiotic rat 56 days after ligation and severance of the common bile duct. The pedicle had ceased to function a short time before death, with consequent jaundice present when the rat died spontaneously. The photograph shows hyperplastic bile ducts, replacing most of the hepatic cells. A few remaining groups of hepatic cells with hyperchromatic nuclei are seen.

Fig 2—Same liver magnified 556 times.

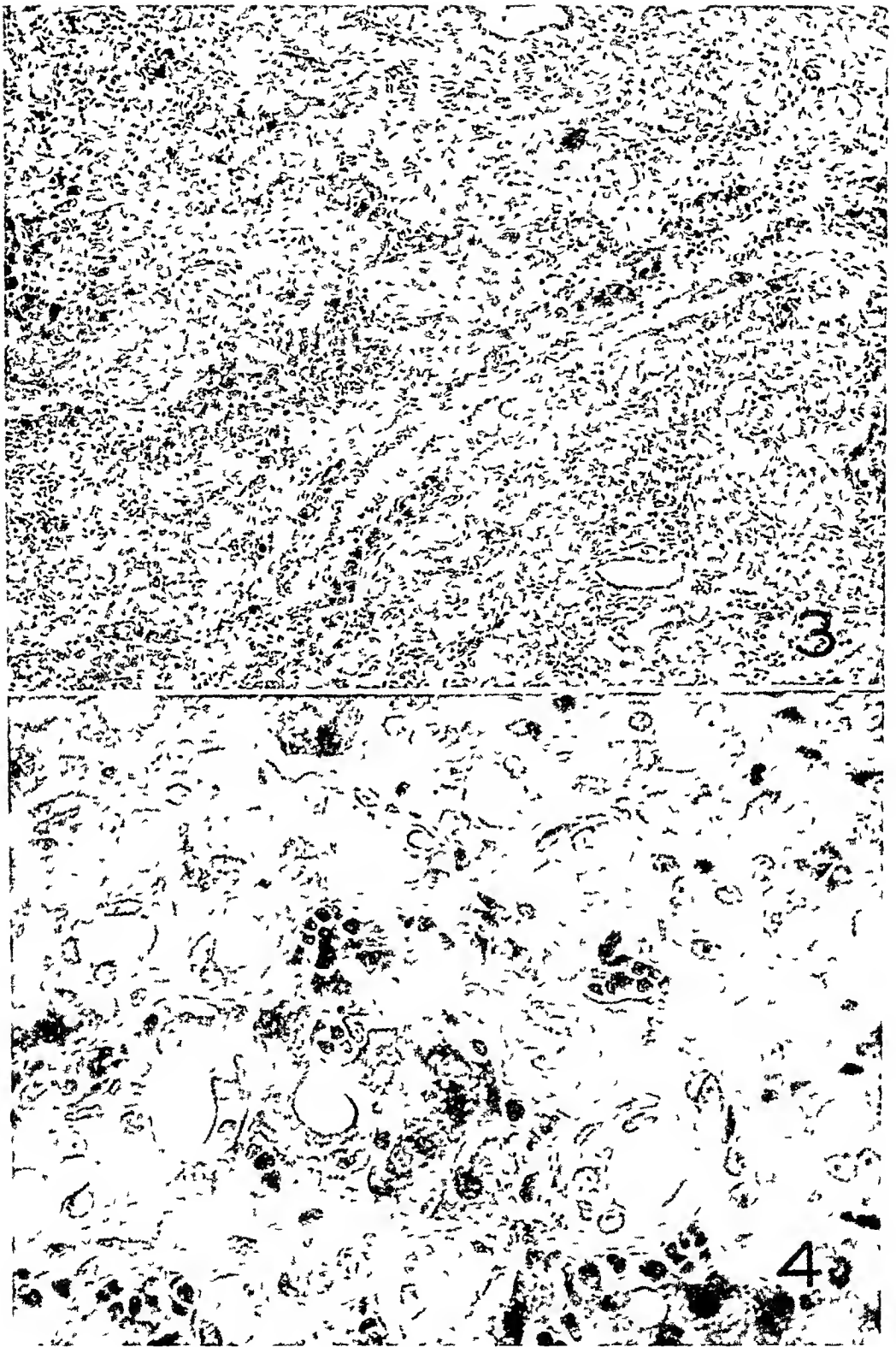


Fig 3—Liver ( $\times 122$ ) of a parabiotic rat 107 days after ligation and severance of the common bile duct. The pedicle was functioning adequately. No jaundice was present when the rat died with pulmonary infection. The photograph shows actively proliferating bile duct epithelium replacing many hepatic cells. The few darkly staining cells in the photograph are hepatic cells.

Fig 4—Same liver magnified 556 times.

there were no widespread necrotic areas in which a number of cells showed disintegrating cytoplasm and nuclear fragmentation. Only occasionally were there small isolated areas of this character. The disappearance of hepatic cells seemed to have occurred by a slow rather than a rapid process.

The colorless fluid in the dilated large ducts indicated that the bile pigment had not entered the large ducts and apparently had not entered the small duct ramifications. Perhaps such accumulation represents passing of fluid from the blood stream into the lumen of the duct without passing through hepatic cells.

The pallor of the liver in the late stages and the absence of bile, histologically determined, indicate that the disappearance of hepatic cells can hardly be ascribed to injury caused by retained bile. This point of view is in agreement with the opinion expressed by Moore. The histologic appearance suggests that the proliferating duct epithelium had encroached on hepatic cells and had caused their disappearance by pressure atrophy or nutritional atrophy. That is, gradual atrophy, rather than acute necrosis, seems to be the process leading to disappearance of hepatic cells.

One can merely speculate on the factors which determine this extensive proliferation of duct epithelium. There is no need of reviewing the theories expressed in the extensive literature on obstruction of bile ducts in the single animal, because in the case of the jaundiced animal retention of bile probably plays an important role in the injury of the hepatic cells, which in turn may lead to hyperplasia of bile ducts. In the present experiments, since extensive necrosis did not occur, substances released from disintegrating cytoplasm are probably not factors inducing proliferation. However, it may be that the slow disappearance of hepatic cells by pressure of distended ducts releases substances which induce hyperplasia of duct epithelium. In the rat, since there is no gallbladder for absorption, the factor of distention is of importance. One wonders whether the physical tension of a distended duct may in some other way lead to cellular proliferation. No really adequate explanation is apparent.

Experiments of this type may prove to be a convenient method of producing long-sustained hepatic inadequacy in order to study various altered functions.

#### SUMMARY

Experiments were made in which the common bile duct was cut between ligatures in a rat which was in parabiosis with a litter mate whose common bile duct was unobstructed.

The experiments demonstrated the extreme proliferation of bile duct epithelium that can be maintained for periods up to 114 days, this constituting a much longer period than any during which the proliferation could be maintained in single jaundiced rats.

# RIGHT AORTIC ARCH WITH A VASCULAR RING CONSTRICTING ESOPHAGUS AND TRACHEA

Report of Two Cases

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**A**NOMALIES of the aortic arch amenable to correction have gained in importance since the advent of modern technics in thoracic surgery<sup>1</sup> Not all the anomalies of the aortic arch produce clinical manifestations<sup>2</sup> These occur usually when a vascular ring exists, a portion of which lies dorsal to the esophagus Constriction of the esophagus and the trachea enclosed in the ring is manifested clinically by dysphagia, dyspnea and cyanosis The vascular ring also presents characteristic roentgenologic appearances<sup>3</sup>

A vascular ring may constrict the esophagus and the trachea in association with a double aortic arch,<sup>4</sup> or with a left retroesophageal arch,<sup>5</sup> or with a right retroesophageal arch<sup>6</sup> In this paper we report the clinical histories and the necropsy observations of 2 infants, each with a right aortic retroesophageal arch forming a vascular ring which constricted the esophagus and the trachea In one of these the anomaly was discovered at necropsy, in the second it was recognized clinically

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From the Departments of Pathology and Pediatrics, University of Oklahoma School of Medicine, and the University of Oklahoma Hospitals

1 Gross, R E Surgical Treatment for Abnormalities of the Heart and Great Vessels, Springfield, Ill, Charles C Thomas, Publisher, 1947

2 Eisen, D Radiology **42** 570, 1944

3 Arkin, A Am Heart J **11** 444, 1936

4 Gross, R E, and Ware, P F Surg, Gynec & Obst **83** 435, 1946  
Potts, W J, Gibson, S, and Rothwell, R Arch Surg **57** 227, 1948

5 Edwards, J E Proc Staff Meet, Mayo Clin **23** 108, 1948 Paul, R N J Pediat **32** 19, 1948

6 Bedford, D E, and Parkinson, J Brit J Radiol **9** 776, 1936 Brenster, R, Herlihy, W F, Lawson, D W, and Nowland, R J M J Australia **1** 47, 1942 Wurtz, K G, and Powell, N B J Pediat **33** 722, 1948

## REPORT OF CASES

CASE 1—M A, a 7 month old Mexican girl, was first seen at the University of Oklahoma Hospitals, June 27, 1947, at the age of 7 weeks, because of bilateral clubfeet (talipes equinovarus). At this time it was noted that she had a temperature of 103 F and rales in both lungs. Roentgenologic examination disclosed an enlarged cardiac shadow. Electrocardiographic examination revealed right cardiac hypertrophy, which was interpreted as due to congenital heart disease, the type undetermined. The patient was treated for pneumonia with penicillin and oxygen. Casts were applied for correction of the deformity of the feet. On July 17 she was discharged, to be followed in the outpatient department. She was again admitted on September 26 and was treated with penicillin and digitalis. She was discharged improved, October 4, to the Convalescent Home. Occasional spells of cyanosis occurred, respiratory difficulty developed and the patient was admitted for the third time on November 19. She was discharged December 5, with a maintenance dose of digitalis. She continued to have cyanosis and dyspnea, and edema developed. Two days prior to her last admission, December 29 she seemed to choke when taking the formula feeding and began to refuse feedings.

At the time of this last admission the patient was underdeveloped, poorly nourished and obviously in acute distress. Respirations were rapid. She had a frequent productive cough, sucked her thumb vigorously and refused feeding as if afraid of it. Rhonchi and coarse rales were heard over both lung fields. The heart was enlarged to percussion. A systolic murmur was heard, loudest in the second and third intercostal spaces to the left of the sternum. The cardiac rate was rapid, the rhythm was regular. The liver extended 4 cm below the right costal margin. There was pitting on pressure of the feet and ankles. The red blood cell count was 3,870,000, the hemoglobin content, 10 Gm. The white blood cell count was 10,600, with neutrophilic granulocytes 87, lymphocytes 12 and monocytes 1 per cent. Urinalyses gave essentially negative results. Repeated roentgenologic examinations revealed cardiac enlargement, interpreted as congenital heart disease, and varying degrees of pulmonary congestion and infiltration.

The patient was placed in an oxygen tent, given digitalis, penicillin, sulfadiazine and hypertonic solution of dextrose. Her condition did not improve and she died December 31, the third day after admission.

*Necropsy* (twenty-fours after death).—The body was 64 cm long and weighed 4,850 Gm. Malnutrition was evident. The skin varied from pale to white. There was pitting on pressure of the lower extremities. The peritoneal, pleural and pericardial cavities contained no excess fluid. The heart measured 6.5 cm from base to apex and 6 cm across the base. The apex was rounded and bifid and was made up almost equally of the two ventricles. The superior and the inferior vena cava opened into the right atrium as usual. No anomalies of the coronary sinus and its tributaries were noted. The topographic relation of the pulmonary artery was as usual. The coronary arteries arose from the bulb of the aorta and were of usual size and distribution. The ascending portion and arch of the aorta curved over the right main bronchus and descended on the right of the esophagus (fig 1). From the arch arose first the left common carotid artery, which crossed anterior to the trachea to ascend on the left side, then the right common carotid artery, and finally the right subclavian artery. Next a diverticulum (remains of the left fourth arch) arose

from the medial surface of the aorta and curved behind the esophagus. From the diverticulum came off the left subclavian artery. Continuous with the diverticulum was the obliterated ductus arteriosus that connected with the left branch of the pulmonary artery. Thus a vascular ring surrounded the esophagus and the trachea causing constriction of both. The cardiac orifices and valves were of usual size and appearance. The foramen ovale was partially covered by a delicate membrane, leaving an opening 1 by 0.4 cm. The inter-ventricular septum had an opening 0.8 cm in diameter in its membranous portion. The lungs were air-containing anteriorly, and were non-air-containing, firm and somewhat lumpy posteriorly. On the inner surface of the upper lobe of the right lung was an azygos lobe, 2 by 1.8 cm. There were other accessory fissures in both lungs. Except for chronic passive congestion of the viscera there were no other pertinent changes.

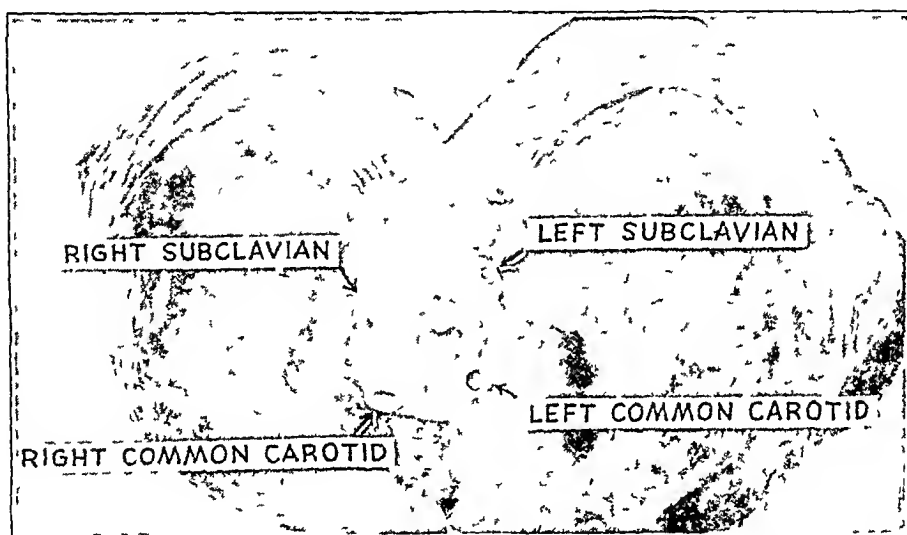


Fig 1 (case 1)—Right aortic retroesophageal arch forming a vascular ring which constricted the esophagus and the trachea of a Mexican girl aged 7 months

CASE 2—M W, a 5 month old Negro girl, was admitted, Dec 1, 1948, because of choking, vomiting, respiratory difficulty and inadequate gain in weight. She was born spontaneously at term and weighed 5 pounds and 8 ounces (2,494.5 Gm). She was breast fed for three months, then was bottle fed. When she was about 6 weeks old, respiration became wheezing and a frequent nonproductive hacking cough appeared. At the age of 10 weeks she began to have episodes of crying, cyanosis and perspiration. The attacks occurred three to six times daily, ending with rapid, labored respirations, exhaustion and vomiting or defecation. Feeding of 2 ounces (57 cc) caused regurgitation and at times initiated the attacks. Sleeping in an upright position seemed to lessen the severity of wheezing and coughing and the frequency of the episodes.

At the time of admission the patient was poorly developed and poorly nourished. She cried continually, coughed intermittently and had an expiratory grunt. Her temperature was 99.8 F. There was substernal retraction with inspiration.



The lung fields were clear to auscultation and percussion. The cardiac rate was rapid. A loud systolic murmur was heard over the pulmonic and mitral valve areas and was not transmitted. There was no palpable thrill. The liver



Fig 2 (case 2)—*A* and *B*, roentgenograms showing the esophagus constricted at the level of an anomalous vascular ring in a Negro girl aged 5 months. *A* is the posterior-anterior, and *B* the right lateral, view. *C*, right aortic retroesophageal arch forming a vascular ring which constricted the esophagus and the trachea.

extended 1 cm below the right costal margin. The red blood cell count was 4,910,000, the hemoglobin content, 10.5 Gm. The white blood cell count was

14,400, with neutrophilic granulocytes 38, lymphocytes 60 and monocytes 2 per cent. Urinalysis gave essentially negative results. The Mazzini test of the blood was negative. An intradermal test with tuberculin diluted 1:1,000 produced a negative reaction. Roentgenograms of the chest revealed the heart to be moderately enlarged and the costophrenic angles and lung fields clear except for accentuation of the hilar vascular markings. Roentgenologic examination after barium sulfate was swallowed revealed narrowing of the upper end of the esophagus in the region of the great vessels and a pressure defect on the right side of the esophagus (fig 2A and B). This was interpreted as a right aortic arch with a vascular ring. The patient was given penicillin and digitoxin, and other supportive measures were used. She had several episodes of tachycardia, respiratory difficulty and cyanosis. On December 9, at 3:30 p. m., she had a sudden severe episode of respiratory embarrassment. Following this, immediate operation seemed indicated, though the patient's condition was not favorable. At 11:30 p. m. an exploratory thoracotomy was made by Dr. J. Moore Campbell. A large artery that arose from the aortic arch, crossed the trachea and ascended on the left side of the latter was ligated. Following ligation, the trachea and the esophagus seemed under less tension. The patient's condition, however, remained critical, and she died on December 10, eight hours after the operation.

*Necropsy* (three hours after death).—The body was 55 cm. long and weighed 2,975 Gm. The skin was pale brown. The chest appeared enlarged in its ventro-dorsal diameter and was 32 cm. in circumference. A sutured incision, 8 cm. long, extended from the sternum between the second and third ribs to the left posterior axillary line. There was no excess fluid in the serous cavities. Minute focal areas of extravasation of blood were noted in the epicardium. The heart measured 5 cm. from base to apex and 5.5 cm. across the base. The apex was rounded and made up almost equally of the two ventricles. The right atrium and its auricle were distended. The superior and the inferior vena cava opened into the right atrium as usual. No anomalies of the coronary sinus and its tributaries were noted. The pulmonary artery and the aorta were of nearly equal size. The ascending portion of the aorta arched over the right main bronchus and passed down to the right of the esophagus (fig 2C). From the arch arose three arteries, first, the left common carotid (ligated), second, the right common carotid and, third, the right subclavian. The left subclavian artery arose from an aortic diverticulum. The diverticulum lay behind the esophagus and continued into the closed ductus arteriosus which connected with the left branch of the pulmonary artery. The arterial ring thus formed encircled the esophagus and the trachea. The cardiac orifices were proportionate, and their valves were delicate. There was an opening, 1 by 0.3 cm., in the membranous portion of the interventricular septum. Both lungs contained air in their anterior portions and were firm, dark red-blue and lumpy in their posterior portions. Except for chronic passive congestion of the viscera no pertinent changes were seen in any of the organs.

#### COMMENT

Dysphagia, dyspnea and cyanosis when observed in an infant should cause suspicion of the presence of one of the aortic arches with a vascular ring. This may be verified or excluded by appropriate roentgenologic examination. In our first case, a vascular anomaly was

not suspected, and no roentgenologic examination of the esophagus was made, though the presence of a cardiac anomaly was inferred. In the second case, the clinical diagnosis was established, and surgical intervention to relieve the pressure on the esophagus and the trachea was attempted.

#### SUMMARY

Two infants, each with a right retroesophageal aortic arch constricting the esophagus and the trachea, have been observed clinically and at necropsy. The characteristic clinical manifestations were present in both, and in one the condition was recognized while the child was still alive.

# ACUTE LESIONS IN WEANLING RATS FED 4-AMINOPTEROYLGLUTAMIC ACID

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THE INHIBITORY action of 4-aminopteroylglutamic acid on growth and blood cell formation has been reported by Minnick and Moore<sup>1</sup> for the guinea pig, by Franklin and co-workers<sup>2</sup> for the mouse and by Oleson and associates<sup>3</sup> for the chick, the rat and *Streptococcus tectalis* R. The effects of this antivitamin on the Rous chicken sarcoma have been studied by Woll<sup>4</sup> and by Little and co-workers<sup>5</sup>. Several cases of remission of acute leukemia in children treated with this compound have been presented by Farber and associates<sup>6</sup>.

In this paper we wish to report on the histologic changes observed in weanling rats fed this potent antagonist of pteroylglutamic acid. It must be emphasized that these are acute experiments, since the doses of the antagonist used produce rapid loss of weight, severe emaciation and death in three to five days.

## EXPERIMENT

Typical data on the inhibition of growth produced by the antagonist, 4-aminopteroylglutamic acid, and on its reversal by pteroylglutamic acid have been presented previously<sup>3</sup>, so the details of the feeding experiments will not be repeated here. An identical experiment was set up to gain information concerning the sequence of the reactions produced in the tissues. A control group of weanling rats weighing about 40 Gm was fed our basal synthetic diet, and two groups were given in addition 10 and 50 micrograms of 4-aminopteroylglutamic acid orally per day for five days. Two animals from each group were killed at twenty-four hours.

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From the Lederle Laboratories Division, American Cyanamid Company

1 Minnick, V, and Moore, C V. *Federation Proc* **7** 276, 1948

2 Franklin, A L, Stokstad, E L R, and Jukes, T H. *Proc Soc Exper Biol & Med* **67** 398, 1948

3 Oleson, J J, Hutchings, B L, and Subbarow, Y. *J Biol Chem* **175** 359, 1948

4 Woll, E. *Tr New York Acad Sc* **10** 83, 1948

5 Little, P A, Sampath, A, Paganelli, V, Locke, E, and Subbarow, Y. *Tr New York Acad Sc* **10** 91, 1948

6 Farber, S, Diamond, L K, Mercer, R D, Sylvester R F, Jr, and Wolff, I A. *New England J Med* **238** 787, 1948

intervals in the course of five days. The nature of the tissue changes produced was determined by histologic examination. Dead or moribund animals were discarded.

#### HISTOLOGIC METHODS

All tissues were placed in 10 per cent formaldehyde in 90 per cent ethyl alcohol within ten minutes after the animal had been killed with ether. Graham's stain (peroxidase reaction) was applied to all tissues by our previously described method.<sup>7</sup> Paraffin sections were cut at 4 microns and stained by the hematoxylin-eosin technic.

#### MICROSCOPIC OBSERVATIONS OF THE CONTROL GROUP

The thymus, liver, kidney, adrenal gland, stomach and rectum were histologically normal, with no alterations in the five day period. The spleen on the first day showed considerable hemopoiesis and a few megakaryocytes. This we consider to be an embryonic type which gradually changes to the adult type by the fifth day. This is consistent with the established histology of our strain.

#### MICROSCOPIC OBSERVATIONS OF THE TREATED GROUPS

The spleen, the thymus and the bone marrow showed pronounced effects of the 4-aminopteroylglutamic acid used in this acute experiment. The higher level of the antagonist differed from the lower only in effecting an earlier maximum change.

*Spleen*—At the end of the first twenty-four hours the connective tissue stroma was prominent, giving an accentuated line of demarcation to the malpighian corpuscles. In the succeeding days the fibrous changes increased with disappearance of most of the normal pulp and nodule cells. By the end of the fourth day the entire organ was fibrous. The malpighian corpuscles showed degenerating small lymphocytes, the number increasing daily until but few normal ones were present in the spleen. The large lymphocytes were decreased in number, yet a few still remained among the stromal fibers. Individual variation occurred in the damage of the spleen, but the change in structure of the entire organ was always striking, as was the decrease in small lymphocytes (fig 1).

*Thymus*—The progressive changes occurred in definite order. As the capillaries dilated, the prominence of the connective tissue stroma increased, the Hassall corpuscles increased in number, and the thymocytes of the cortex degenerated. By the end of the fourth day the normal structure of the gland was replaced by fibrous stroma in which there were only degenerating lymphocytes with pyknotic nuclei. This reaction paralleled the changes in the spleen (fig 2).

*Femur*—The marrow was rapidly depleted. The changes began on the first day. The immature red and white cells decreased first, the youngest more rapidly. Finally the mature red and white cells decreased until the marrow became virtually aplastic. The eosinophilic and basophilic granulocytes decreased in number also. Mitoses were not present. Megakaryocytes were rarely seen. The hemopoietic areas were replaced by connective tissue stroma, hemorrhage and dilated capillaries (fig 3).

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<sup>7</sup> Ritter, H. B., and Oleson, J. J. Arch Path 43:330, 1947

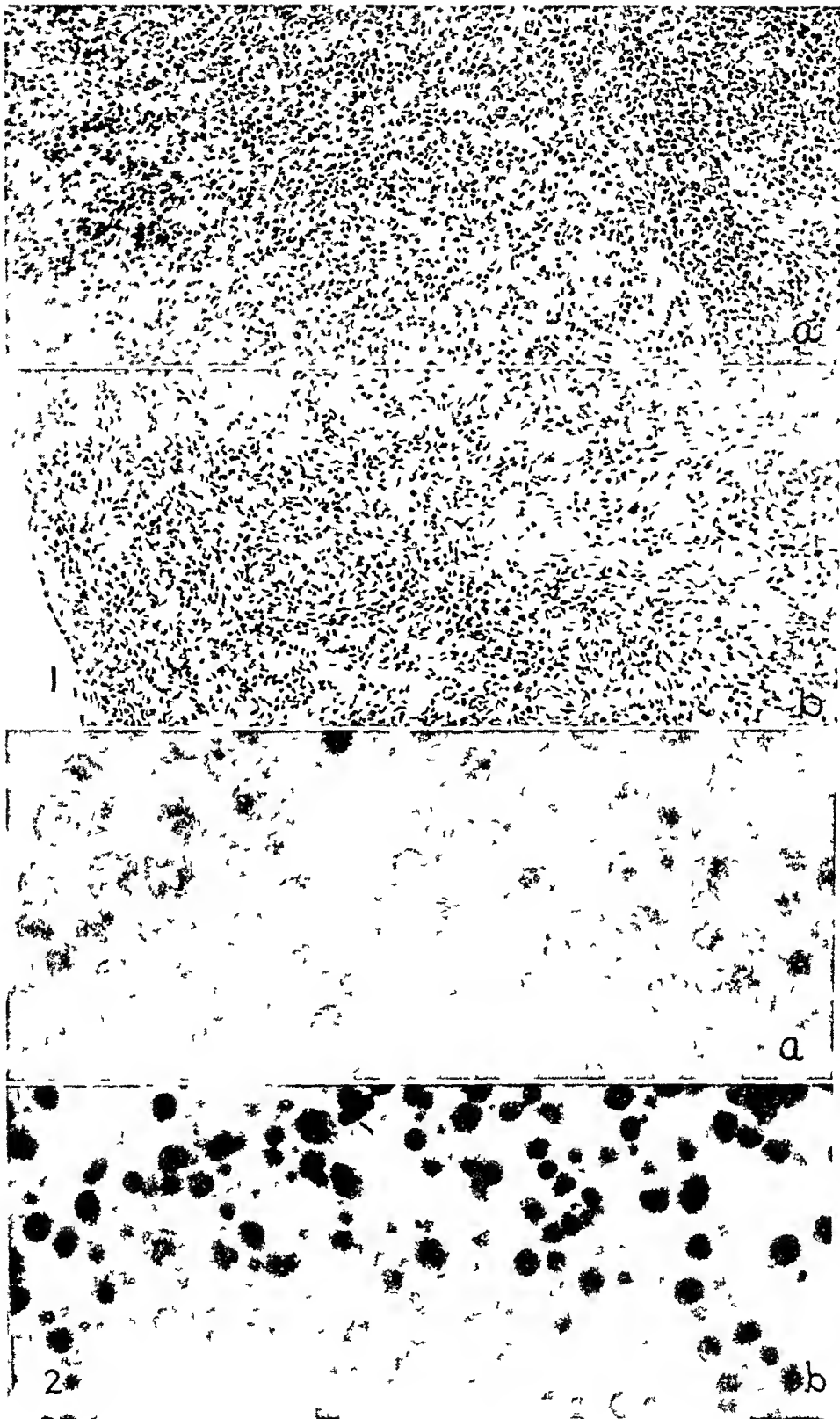


Fig 1—Spleen ( $\times 180$ , hematoxylin-eosin) (a) control, (b) weanling rat given 50 micrograms of 4-aminopteroylglutamic acid per day for five days. Note the loss of structure and splenic cells in *b*.

Fig 2—Thymus,  $\times 1,100$ , hematoxylin-eosin (a) control, (b) weanling rat given 50 micrograms of 4-aminopteroylglutamic acid per day for five days. Note the degeneration of cortex in *b*.

Since the younger marrow cells disappeared rapidly and were not replaced, the mature cells predominated. As these entered the blood stream the marrow became depleted. The peripheral counts showed no aberrations during the five day experiment.

*Liver, Kidney, Adrenal Gland, Gastrointestinal Tract*—The dilatation of the capillaries and sinusoids was prominent in these tissues. Cellular changes, such as hypertrophy of nuclei and/or cytoplasm, loss of cytoplasmic granules and loss of staining power occurred infrequently and were difficult to evaluate in these short term experiments.

*Thyroid Gland, Pancreas, Gonads, Heart Muscle*—No microscopic changes were detected in these tissues.

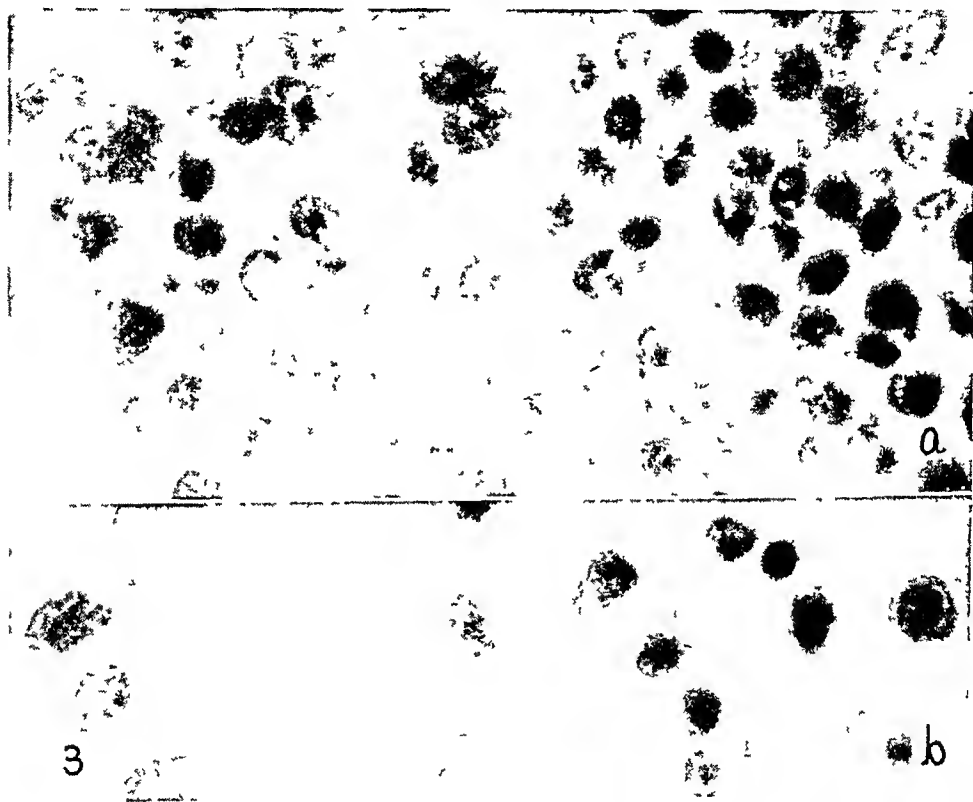


Fig 3—Femur,  $\times 1,100$ , hematoxylin-eosin (a) control, (b) weanling rat given 50 micrograms of 4-aminopteroylglutamic acid per day for five days. Note the degeneration of cells in b.

#### PREVENTION OF TISSUE CHANGES

Sections from our previous experiments<sup>3</sup> showed that synthetic pteroylglutamic acid when given at thirty to forty times the level of the antagonist will completely prevent the changes described.

#### SUMMARY

Low doses of 4-aminopteroylglutamic acid given orally or intramuscularly to weanling rats produced an immediate loss of weight,

severe emaciation and death in three to five days. High doses of pteroylglutamic acid permitted survival and partially overcame the inhibition of growth.

Marked pathologic changes were seen in degeneration of small lymphocytes in the spleen and in the thymus, accompanied by progressive fibrous changes from the first day until death, and nearly complete aplasia of all cellular elements of the marrow, with hemorrhage and fibrous changes.

Histologic study of kidney, adrenal gland, liver and gastrointestinal tract showed only questionable changes. No changes of the histologic aspects of other tissues were seen.



# EFFECTS OF THE ADMINISTRATION OF A VITAMIN E CONCENTRATE AND OF CHOLESTEROL AND BILE SALT ON THE AORTA OF THE RAT

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LOS ANGELES

THE influence of vitamin E (alpha tocopherol) on cholesterol metabolism and atherosclerosis is still obscure, and reports in the literature are contradictory. According to Monnier and associates,<sup>1</sup> hypervitaminosis-E had no significant effect on total blood cholesterol in the rat, and Dam<sup>2</sup> observed that *d,l*-tocopherol acetate did not modify the aortic cholesterol deposition of rabbits or chicks fed diets high in cholesterol. Morgulis and Spencer,<sup>3</sup> on the other hand, reported a significant rise of blood cholesterol in rabbits fed a vitamin E-deficient diet, while Bruger<sup>4</sup> found that vitamin E markedly increased the cholesterol content of the aorta of the rabbit fed a high cholesterol diet.

In view of these contradictory results the relation of vitamin E to cholesterol metabolism was investigated. The present report deals with the effects of avitaminosis-E and those of high doses of a vitamin E concentrate on cholesterol metabolism and atherosclerosis in the rat.

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The authors wish to express their appreciation for the use of the facilities of the Hancock Foundation.

Contribution no 195 from the Department of Biochemistry and Nutrition, University of Southern California.

1 Monnier, M., Farchadi, A., and Maulbetsch, A. *Compt rend Soc phys hist nat. Geneve* **58** 244, 1941, *Chem Abstr* **38** 2379, 1944.

2 Dam, H. *J Nutrition* **28** 289, 1944.

3 Morgulis, S., and Spencer, H. *C. J Nutrition* **12** 173, 1936.

4 Bruger, M. *Proc Soc Exper Biol & Med* **59** 56, 1945.

## EXPERIMENTAL PROCEDURES

Nursing rats were restricted to a diet deficient in vitamin E<sup>5</sup>. The young rats were fed the same diet until, a few days after weaning, the litters were divided into four experimental groups of 4 males and 4 females each, as shown in the table. Litter mates were distributed evenly as far as possible.

Groups 1 and 2 were fed the deficient diet without supplement (E-deficient). For groups 3 and 4 (E-high) the diet was supplemented once weekly with 150 mg of a vitamin E concentrate prepared from vegetable oils and containing 34 per cent "mixed tocopherols"<sup>6</sup>, the concentrate was fed with a syringe, after being diluted with ethyl laurate. Somewhat older rats fed this laboratory's stock diet served as controls (groups 5 and 6). For groups 2, 4 and 6 (E-deficient, C + B) cholesterol<sup>7</sup> was added to the diet at a level of 1 per cent, and, in order to increase absorption of cholesterol, 0.5 per cent of bile salt was added.

Body weights were noted during the experimental periods, which varied from twenty to twenty-seven weeks. At autopsy total cholesterol levels were determined for blood and various tissues, a modified Schoenheimer-Sperry-Chaney procedure being used<sup>10</sup>.

The aorta was cut open lengthwise and fixed in formaldehyde solution. Sample strips, especially of the thoracic portion, were embedded in gelatin and examined as frozen sections stained with Nile blue sulfate. Interesting specimens were also stained with other dyes, gelatin and paraffin sections being used<sup>11</sup>.

Cholesterol was identified by its birefringence with the help of a pair of polaroid films<sup>12</sup> inserted in the microscope. To distinguish small cholesterol deposits from other anisotropic material, such as collagenous fibers, the slides were examined before and after extraction with chloroform. Pairs of photomicrographs were also made in this manner. The work was rendered difficult because, on storage, fixed tissues and sections slowly lose by diffusion part of their stainable fat and cholesterol.

## RESULTS

*Body Weight*—At an age of approximately 80 days, the weight curves of the vitamin E-deficient animals began to level off. During the following month the male rats of group 1 (E-deficient) lost an average of about 40 Gm and were in such poor general condition that they were killed and examined. At that time, respiratory infections and lesions of the skin were widespread, but

5 The diet was made up as follows: 1,200 Gm of sugar, 800 Gm of starch, 460 Gm of lard, 800 Gm of a commercial casein, 320 Gm of yeast (strain G), 300 Gm of cellulose flour (cellufLOUR®), 120 Gm of salt mix, 12 Gm of a vitamin D (calciferol) concentrate (drisdol®), 1 mg of carotene.

6 The concentrate is marketed by Distillation Products, Inc.

7 Dr. F. Fenger, of the Armour Laboratories, and Mr. Noble F. Payton, of the Suburban Chemical Company, Chicago, supplied some of the cholesterol used.

8 Schoenheimer, R. *Biochem. Ztschr.* **147**: 258, 1924.

9 Extract of ox bile U. S. P., both Wilson's and Armour's products were used; Dr. F. Fenger of the Armour Laboratories, supplied some of the bile salt used.

10 A description of this modification of the Schoenheimer-Sperry-Chaney procedure is to be published.

11 The Department of Anatomy of the University of Southern California School of Medicine lent two microtomes for our use.

12 Polaroid films are manufactured by the Polaroid Company, Cambridge, Mass.

only 1 rat exhibited muscular symptoms. The animals of group 3 (E-high) grew at a normal rate.

The influence on body weight of dietary cholesterol and bile salt was not uniform. A beneficial effect was observed in the male rats of group 2 (E-deficient C + B). The female animals of this group, however, gained significantly less than their litter mates on the same diet without cholesterol and bile salt. In group 4 (E-high, C + B) the male rats fed the high cholesterol diet gained significantly less weight than their respective litter mates, whose diet was not supplemented with cholesterol and bile salt. The female rats of this group, however, were not much affected.

*Sex Organs*—The rats of group 1 (E-deficient) showed the expected genital symptoms. Addition of cholesterol and bile salt to the diet (group 2) aggravated these defects. The male animals of group 3 (E-high) also appeared

*Effect of Vitamin E Deficiency as Influenced by the Administration of a Vitamin E Concentrate, Cholesterol and Bile Salt*

(Feeding Periods 20 to 27 Weeks)

| Group | Vitamin E<br>Concen-<br>trate,<br>Mg per Wk | Dietary<br>Cholesterol +<br>Bile Salt | Rats   | Plasma<br>Cholesterol,<br>Mg per 100 Cc | Liver<br>Cholesterol<br>per Cent | Histologic Changes of<br>Aorta—Rats Show-<br>ing Given Change |                              |                           |
|-------|---|---------------------------------------|--------|---|----------------------------------|---|------------------------------|---------------------------|
|       |   |                                       |        |   |                                  | Fatty<br>Infiltro-<br>tion                                    | Choles-<br>terol<br>Deposits | Intimal<br>Sele-<br>rosis |
| 1     | None  | None                                  | 7      | 41 ± 4                                  | 0.25 ± 0.02                      | 7   | 0                            | 0                         |
| 2     | None  | C + B                                 | 5      | 155 ± 12                                | 2.55 ± 0.61                      | 5   | 0                            | 0                         |
| 3     | 150   | None                                  | 7      | 56 ± 2                                  | 0.30 ± 0.01                      | 5   | 7                            | 6                         |
| 4     | 150   | C + B                                 | 7      | 122 ± 11                                | 3.68 ± 0.09                      | 4   | 0                            | 0                         |
| 5     | Stock diet                                  | None                                  | 17(7)* | 60 ± 3                                  | 0.21 ± 0.02                      | 3   | 0                            | 0                         |
| 6     | Stock diet                                  | C + B                                 | 5(8)*  | 128 ± 12                                | 2.03 ± 0.64                      | 4   | 0                            | 2                         |

\* The number in parentheses is the number of aortas examined histologically. The animals of groups 5 and 6 were 1 to 2 years old. Group 6 includes 3 rats fed the experimental diet for thirty-eight weeks. Further control rats, from other experiments, were not included in this table, because conditions were slightly different. The aortas of those animals showed the same histologic picture.

sterile, while those of group 4 (E-high, C + B) appeared fertile but had smaller prostates and seminal vesicles.

*Liver*—As was to be expected,<sup>13</sup> the livers of the animals fed cholesterol and bile salt (groups 2, 4 and 6) were pale and enlarged, regardless of the level of the vitamin E intake.

*Tissue Cholesterol Levels*—Total cholesterol was determined for plasma, liver, adrenal glands, kidneys and spleen. As a consequence of the high cholesterol intake, cholesterol was increased in the plasma about two to four fold, in the liver approximately five to ten fold (table 1), and in the adrenal glands about 1.5 to 3 times. The level of vitamin E in the diet did not influence these changes significantly. The cholesterol contents of the kidneys and the spleen were normal except in group 2 (E-deficient, C + B), in which they were increased by about 50 to 100 per cent.

*Morphology of Aorta*—Several of the vitamin E-deficient rats (groups 1 and 2, table) had white fatty plaques in the aorta. On histologic examination,

<sup>13</sup> Anitschkow, N., and Chalatow, S. S., quoted and confirmed by Wacker, L., and Hueck, W. *Arch f exper Path u Pharmacol* 74:416, 1913.

all aortas of these groups had fatty films on endothelium and elastic lamellas and had more stainable fat and foam cells in the endothelium than were found in control material. The foam cells were not birefringent, a species characteristic of rats and mice, as emphasized by Leffkowitz and Rosenberg<sup>14</sup>. The fat imbibition appeared equally high in groups 1 (E-deficient) and 2 (E-deficient, C + B). Sparse cholesterol crystals were seen in the adventitial fat and on the surface of the endothelium. The tissue of the aortic and mitral valves and their base appeared swollen and poor in cells.

The rats fed 150 mg of the vitamin E concentrate once weekly, but no cholesterol and bile salt (group 3, E-high, table), showed overdevelopment of collagenous tissue at the base of the valve and in the medial coat of the aorta. In the intima of the ascending aorta and the dorsal arc there were sclerotic foci, cellular in female, larger and hyalin-like in male, animals. Close by in the media there were often vasa vasorum. The sclerotic foci and the acacia of the surrounding medium were imbued with chloroform-soluble, highly birefringent material, presumably cholesterol ester. With time, this substance was seen to cover an increasingly large area and fade out. The endothelial cells covering the marginal part of the foci and those in other spots were loaded with fat and phospholipids or they were replaced by foam cells. But over the centers of the foci the endothelium had degenerated and only free fat droplets remained.

The rats of group 4 (E-high, C + B), which were fed the same amount of vitamin E concentrate but, in addition, were given cholesterol and bile salt, showed only some vasa vasorum and slight fat infiltration of the endothelium, not exceeding that found in controls. The transparency of the aortic wall was not perceptibly increased after chloroform extraction, indicating a singularly low fat content of the media.

#### COMMENT

Neither vitamin E deficiency nor an excessive dose of the vitamin E concentrate modified cholesterol levels in blood, liver or adrenal glands of rats significantly, in agreement with observations by Monnier and co-workers<sup>1</sup> and Dam<sup>2</sup>. Our findings disagree, however, with results obtained by Morgulis and Spencer,<sup>3</sup> who observed a rise in blood cholesterol of rabbits in muscular dystrophy. Whether the discrepancy is due to the difference in species or to a difference in experimental conditions is not known.

In contrast to the chemical findings, the morphologic examination of the aorta of the vitamin E-deficient animals showed a tendency toward fatty infiltration not accompanied by deposition of cholesterol. This imbibition of fat was correlated neither with the cholesterol levels of blood or liver nor with the cholesterol and bile salt contents of the diet. The phenomenon may be related, however, to the increased permeability of capillaries observed by Dam and Glavind<sup>15</sup> in vitamin E-deficient animals, whether it is also related to Holman's recent finding<sup>16</sup> that vitamin E prevented experimental arteritis in dogs is not known.

<sup>14</sup> Leffkowitz, M., and Rosenberg, D. *Frankfurt Ztschr f Path* **34** 174, 1926.

<sup>15</sup> Dam, H., and Glavind, J. *Nature, London* **143** 810, 1939.

<sup>16</sup> Holman, R. L. *Proc Soc Exper Biol & Med* **66** 307, 1947.

An excess of the vitamin E concentrate proved more harmful than the deficiency. In the group fed an excess of the vitamin E concentrate but no cholesterol plus bile salt, the aorta showed in 6 of 7 animals sclerotic patches which contained cholesterol deposits. These lesions were relatively small and not comparable to those produced, for example, by cholesterol feeding of rabbits or chickens. But it should be stressed that they developed without added dietary cholesterol and that in the males they exceeded in severity the slight pathologic changes of the rat aorta so far produced experimentally by the authors.

No other toxic effect was observed as a result of the high level of the vitamin E concentrate except the mentioned genital symptoms. The animals appeared in a good general condition, and their growth curves resembled those of normal animals.

It has to be emphasized that the vegetable oil concentrate contained 66 per cent of material other than vitamin E and that it is not known at present whether the tocopherols or other components of the concentrate were responsible for the toxic action. As certain oil fractions,<sup>17</sup> in particular, some of the unsaturated fatty acids,<sup>18</sup> are known to cause arterial lesions, and since Holman reported recently on the beneficial effects of vitamin E in the case of arterial lesions produced by renal damage and cod liver oil,<sup>19</sup> it is imperative to repeat the experiment described here with pure tocopherol before further conclusions are drawn.

If it was unexpected to find aortic lesions in the group receiving the high dose of the vitamin E concentrate but no extra cholesterol in the diet, it was even more surprising to observe that a high intake of cholesterol and bile salt completely prevented such damage. The last experimental group, receiving the same, apparently toxic level of the vitamin E concentrate but, in addition, 1 per cent of cholesterol and 0.5 per cent of bile salt with the diet, showed only mild fatty infiltration but neither sclerosis nor cholesterol deposition in the aorta. It is not known whether the cholesterol or the bile salt was responsible for the protective action. It is interesting, in connection with this observation, that Holman reported recently that arterial lesions produced by renal damage and cod liver oil could be prevented or retarded by dietary cholesterol.<sup>19</sup>

#### SUMMARY

The influence of vitamin E (alpha tocopherol) on cholesterol metabolism and the development of arteriosclerosis was investigated in

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17 Holman, R. S., and Swanton, M. C. *Proc. Soc. Exper. Biol. & Med.* **63** 87, 1946.

18 Mylon, E., and Smith, E. R. *Arch. Path.* **45** 21, 1948.

19 Holman, R. S. *Federation Proc.* **5** 223, 1946.

the rat Neither vitamin E deficiency nor an excess of a vitamin E concentrate modified blood or tissue cholesterol levels Avitaminosis E caused fatty infiltration of the wall of the aorta, regardless of the cholesterol content of blood or diet High doses of a vitamin E concentrate produced localized sclerosis and deposition of cholesterol in the aorta These changes were prevented by high dietary cholesterol and bile salt

# THROMBOTIC THROMBOPENIC PURPURA CAUSED BY IODINE

Report of a Case

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AND

JOSEPH SEIFTER, M.D.

PHILADELPHIA

**P**URPURA following iodine treatment has rarely been described. Davis and Saunders<sup>1a</sup> discovered 6 reports in the literature, published from 1877 to 1904, we found 8 cases published between 1919 and 1946<sup>1a-f</sup>. The case to be reported here differs from the previous ones in that the patient died from the rare and uniformly fatal type of acute purpura that is characterized by thrombopenia and widespread formation of thrombocytic clots in the capillaries<sup>2</sup>. It is presented because it gives a possible clue to the genesis of this disease.

## REPORT OF CASE

The patient was an obese Negro woman 24 years of age. Five days before she died she fell acutely ill with diffuse abdominal pain, hematemesis, epistaxis and hematuria. There were petechial hemorrhages in the skin of the chest and in the mucous membranes of the soft palate, the pharynx and the cervix uteri. Blood was oozing from the gums, and gross blood was present in the rectal groove. A tourniquet test was positive. A slight icteric tinge was present. Urticarial lesions were not observed.

Chemical analysis of the blood revealed 8.3 Gm. of hemoglobin per hundred cubic centimeters. A hematocrit reading was 24 per cent. A blood smear showed absence of thrombocytes. A study of the bone marrow (Dr. E. Mertens) revealed a slight increase in cellularity due to an increase in rubriblasts, prorubricytes and rubricytes. There was a slight increase in the number of megakaryocytes, while thrombocytes were remarkably few, most of them staining poorly and showing signs of disintegration. One small blood vessel contained a thrombus. Bleeding time and coagulation time were not determined.

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From the Philadelphia General Hospital, the Wyeth Institute of Applied Biochemistry and the Graduate School of Medicine of the University of Pennsylvania.

1 (a) Davis, W. C., and Saunders, T. S. *Arch. Dermat. & Syph.* **53**: 644, 1946. (b) Osler, W., cited by Jackson<sup>1c</sup>. (c) Jackson, A. S. *J. A. M. A.* **96**: 38, 1931. (d) Toland, C. G., and Askey, J. M. *Proc. Staff Meet., Mayo Clin.* **6**: 597, 1931. (e) Denmg, H. *Munchen med. Wchnschr.* **80**: 562, 1933. (f) Pelner, L. *J. Lab. & Clin. Med.* **27**: 1150, 1942.

2 (a) Baehr, G., Klemperer, P., and Schiffrin, A. *Tr. A. Am. Physicians* **51**: 43, 1936. (b) Trobaugh, F. E., Markowitz, M., Davidson, C. S., and Crowley, W. F. *Arch. Path.* **41**: 327, 1947.

Catheterized urine showed an acid reaction, albumin (2 plus), sugar (3 plus) and 10 to 15 erythrocytes and 5 to 6 leukocytes per high power field. The blood urea nitrogen was 85 mg per hundred cubic centimeters on the third day and 188 mg on the last day of her illness. Carbon dioxide-combining power was 45 volumes per cent.

It was known that the patient had taken kelpidine,<sup>®</sup> a commercial reducing agent containing kelp and iodine, during the last three weeks before she fell ill and that she had also used this drug a year earlier. That this was the cause of her disease, however, was not suspected.

*Postmortem Observations*—The autopsy (Dr D S Pocock) revealed petechial hemorrhages over the chest, the abdomen and the left arm. There were 100 cc of a dark bloody fluid in each pleural cavity and 75 cc of a similar fluid in the pericardial and peritoneal cavities. The endocardium of the auricles, the surface of the kidneys, the intestine and the brain showed numerous petechiae and ecchymoses. The myocardium was diffusely hemorrhagic in places. There was marked congestion in the lungs, the liver, the spleen and the intestine.

Microscopic examination of the various tissues revealed numerous thrombocytic clots in the capillaries and small arteries, especially in those of the following organs: the heart, liver, kidneys, adrenal glands, intestine, lymph nodes and bone marrow. There were small hemorrhages in almost all organs. The

*Milligrams of Iodine per Gram of Moist Tissue*

| Analysis | Kidney | Liver | Lung  | Heart |
|----------|--------|-------|-------|-------|
| 1        | 0.323  | 0.760 | 0.503 | 0.295 |
| 2        | 0.314  | 0.745 | 0.500 | 0.285 |
| 3        | 0.29   | 0.775 | 0.501 | 0.320 |
| 4        | 0.354  | 0.785 | 0.504 | 0.370 |

kidneys contained many protein and hemoglobin casts. There was partial necrosis, particularly of the islets of Langerhans.

The kelpidine<sup>®</sup> tablets which were in the possession of the patient, and some viscera, were analyzed for iodine at the Wyeth Institute by the method of Kendall,<sup>3</sup> which has been found by Wallace and Brodie<sup>4</sup> to be satisfactory for studies of toxicity. The analyses were done by an experienced technician who was engaged in obtaining the iodine content of the tissues of experimental animals which had been subjected to goitrogenic drugs.

The weight of the kelpidine<sup>®</sup> tablets varied from 670.8 to 695 mg per tablet. The manufacturer's label claims 0.2 mg of iodine per tablet. By analysis we found that each tablet contained approximately 0.3 mg of iodine.

Fresh liver, kidneys and lungs from a patient who had not taken iodine were analyzed for comparison. Six samples, ranging from 263 to 493 mg in weight, were taken from each organ. The eighteen determinations made revealed no iodine in these tissues.

The kidneys, the liver, the lungs and the heart of the victim were studied for iodine, four determinations being made on each organ. The amounts of tissue used ranged from 233 to 504 mg. The table shows that the yield of iodine was considerable.

3 Kendall, E. G. J. Biol. Chem. **19**: 251, 1914, **43**: 149, 1920.

4 Wallace, G. B., and Brodie, B. B. J. Pharmacol. & Exper. Therap. **61**: 397, 1947.



The renal values are approximately three to four times those found by Wallace and Brodie<sup>4</sup> in dogs receiving 50 mg of an iodide per kilogram. The hepatic values which we found were considerably higher. This discrepancy can be accounted for by the fact that the patient took the kelpidine® orally. However, from these analytic values one would be tempted to conclude that the patient had taken iodine in addition to the kelpidine® tablets.

# COMMENT

The common cutaneous eruptions following iodine treatment have long been explained as due to idiosyncrasy or to anaphylactic hypersensitivity caused by a combination of iodine and serum protein.<sup>5</sup> Recently Barker and Wood<sup>6</sup> have presented cases showing manifestations of serum disease, namely, fever, eosinophilia, enlargement of lymph nodes and arthralgia, and Rich<sup>7</sup> has described a case in which periarteritis nodosa developed.

Thrombotic thrombopenic purpura has similarly been interpreted as an anaphylactoid reaction. It has been pointed out that capillary hemorrhage and thrombocytic thrombosis are typical of the Schwartzman phenomenon.<sup>8</sup> However, Baehr, Klemperer and Schiffrin<sup>2a</sup> have shown that in purpura the thrombi are on the arterial side of the capillaries, whereas in the Schwartzman phenomenon they are on the venous side. Concerning the possible mechanism of this purpura, it has been postulated by some<sup>9</sup> that it was due primarily to damage of the vascular endothelium. Baehr, Klemperer and Schiffrin<sup>2a</sup> expressed the view that it occurred primarily because thrombocytes were removed from the circulating blood while passing through abnormal capillaries.

In cases of thrombotic thrombopenic purpura due to iodine another possibility suggests itself, namely, that the condition is due primarily to damage of the thrombocytes. It was shown by Stahl<sup>10</sup> that the thrombocyte is iodophilic. When exposed to fumes of iodine crystals it stains weakly yellowish, in some instances showing large mahogany-brown inclusions. Similarly the megakaryocyte shows a light to dark brown cytoplasm containing mahogany-brown bodies, particularly in the periphery of the cell. In view of this observation the possibility of a primary damage of the thrombocytic series deserves serious consideration.

5 Friedberger, E., and Ito, T. *Ztschr f Immunitätsforsch u exper Therap* **12** 241, 1911-1912. Jacobs, J. *J Immunol* **23** 361 and 375, 1932.

6 Barker, W. H., and Wood, W. B. *J A M A* **114** 1029, 1940.

7 Rich, A. R. *Bull Johns Hopkins Hosp* **77** 43, 1945.

8 Schwartzman, G., Klemperer, P., and Gerber, I. E. *J A M A* **107** 1946, 1936.

9 Altschule, M. D. *New England J Med* **227** 477, 1942. Trobaugh and others<sup>2b</sup>.

10 Stahl, R. *Klin Wchnschr* **4** 589, 1925.

It appears to be unlikely that purpura caused by iodine is due to a direct action of the iodine on the thrombocytes or the megakaryocytes, because in this event it would occur more often. It is more likely that here as in other disorders caused by iodine an antigen-antibody reaction is involved. It is conceivable that antibodies evoked by the iodine cause agglutination or disintegration of iodine-laden cells of the thrombocytic series in the circulating blood, and the thrombi formed from these cells are removed from the circulating blood while passing through the capillaries. Since they come with the blood stream, they accumulate on the arterial rather than on the venous side of the capillaries. As a result of the occlusion, the affected endothelial cells suffer from anoxia and become more permeable to the elements of the blood—hence the positive tourniquet test. That this is a reasonable explanation is supported by the fact that in most of the cases reported previously<sup>11</sup> as well as in our case the patient received iodine treatment preceding the course that caused purpura.

#### SUMMARY

A case of fatal thrombotic thrombopenic purpura following iodine medication is reported. It is suggested that the purpura was caused by an antigen-antibody reaction involving cells of the thrombocytic series. The involved cells agglutinated or disintegrated and occluded many capillaries. As a result the blood was depleted of thrombocytes and thrombopenic purpura ensued.

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11 Jackson<sup>1c</sup> Toland and Askey<sup>1d</sup> Dennig<sup>1e</sup>

# RELATIONS BETWEEN VOLUMES OF CLOSED HYPOTHERMAL CEREBRAL LESIONS AND SYMPTOMS IN RABBITS

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AND

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CHICAGO

THIS report describes a method for locally abstracting heat from the brain without disturbing continuity of the skull or introducing complications incidental to mechanical trauma. The excessive thermal outflow leads to a prompt appearance of discrete volumes of edematous, hemorrhagic cerebral tissue. Closed cerebral lesions of this character, controlled with respect to dimensions and location, were reproduced in successive experimental animals. The correlation of the volume per cent of the brain occupied by each lesion with the postoperative clinical course of each animal disclosed data which should prove useful in experimental evaluation of the therapy of acutely expanding intracerebral lesions.

## METHODS

Lesions of the brain were produced by creating a negative thermal gradient from the brain through the intact calvarium to a contiguous instrument cooled by expanding carbon dioxide.

*The Apparatus*—This consisted of two assemblies. One assembly governed the flow of carbon dioxide. The other was the hypothermal instrument by which the dimensions of lesions were controlled. A detailed description of the apparatus has been given elsewhere<sup>1</sup>. The essentials are as follows. Carbon dioxide was conducted to a needle valve at a pressure of 800 to 1,100 pounds per square inch (56 to 710 Kg per square centimeter). When the needle valve was open, carbon dioxide flowed into the hypothermal instrument and expanded proximal to the inner surface of a flat circular metal plate. The metal plate was cooled by the expanding gas. When the plate was placed against the external surface of the skull, heat was abstracted from the successive deepening layers of tissue, namely, periosteum, skull, dura, leptomeninges, cerebral cortex, subcortical white matter and deeper intracerebral structures. The effective negative thermal gradient from the tissue to the plate was almost equal along any line perpendicular to the applied surface of the plate. The effective cross sectional areas of the negative

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1 Hass, G. M., and Taylor, C. B. Arch Path 45:563, 1948.

thermal gradient was, therefore, almost equal to the surface area of the plate in contact with tissue and could be varied by use of plates of different diameters (5 to 26 mm). The effective depths (0 to 13 mm) of the negative thermal gradient were governed by control of the time during which carbon dioxide flowed through the instrument under standard conditions while the cooling plate was in contact with the tissue. The effective volumes (about 0 to 4,000 cu mm) of the negative thermal gradient were sharply defined, therefore, by the diameter of the applied circular cooling plate, the duration of the flow of carbon dioxide and adherence to standard conditions.

*Production of Closed Craniocerebral Lesions*—Experiments were done on albino rabbits, 3 to 6 months of age and weighing 4 to 6 pounds. After anesthesia had been induced with ether, the animal was fastened to the operating table in the prone position. With aseptic precautions a midline incision about 1 inch (2.5 cm) long was made in the scalp over the superior longitudinal suture. The scalp

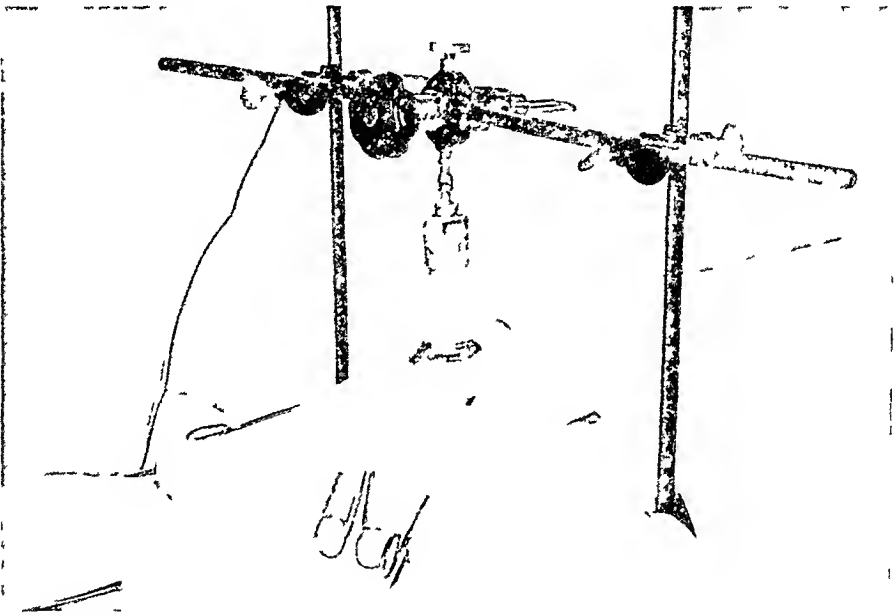


Fig 1—The hypothermal instrument in operation. The hypothermal plate is frozen firmly to the intact external table of the calvarium of a rabbit. Heat is being abstracted from the right cerebral hemisphere within the closed cranial vault. Control of the flow of heat leads to the production of a cerebral lesion of desired volume.

was then reflected laterally to expose the superior surface of the frontal and parietal bones. The cooling plate of desired diameter was screwed into the end of the hypothermal instrument. The surface of the plate was then immersed in a sterile 10 per cent aqueous solution of gelatin at 50°C. The cooling plate was then placed firmly against the skull to the right or to the left of the midline over the part of the cerebrum in which the lesion was to be produced. After the gelatin had made a liquid seal between the cooling plate and the periosteum, and this had been ascertained, excess gelatin was wiped away. Then, by manual control of the needle valve, carbon dioxide at a pressure of 800 to 1,100 pounds per square inch was jetted periodically against the inner surface of the cooling plate (fig 1). The duration of each jet and the interval between jets were timed with a metronome.

at 12 seconds. When a lesion of desired volume had been produced, the instrument and the tissue were allowed to gain heat from the environment. Within five minutes, the tissue and the cooling plate, which had been securely frozen together, spontaneously separated.

Lesions showing a wide range with respect to location, breadth, depth and volume were produced in the superior convexity of the occipital, parietal and frontal lobes of each cerebral hemisphere. Most lesions of great surface area, depth and volume were made in the parietal areas lateral to the median longitudinal fissure. It was easier to reproduce lesions of a desired large volume in this area, because of the contour of the skull.

The dimensions of cerebral lesions were varied by choice throughout a wide range. The diameters varied from 4 to 25 mm, depths from 0.5 to 7 mm and volumes from about 100 to 3,000 cu mm. Depths ranged to all levels through the cortical gray matter and subcortical white matter to the proximal walls of the lateral ventricles and occasionally to the distal walls of these ventricles and structures of the midbrain. Most experiments, however, were devised to define the maximum and the mean survivable volumes per cent of cerebral injury. With this end in view, lethal and near lethal volumes of injury were usually produced in successive animals. No more than two lesions were produced in any animal. When two simultaneous lesions were required, they were usually bilateral.

*Clinical Observations*—Following the production of lesions, periodic observations were made. The time the animal took to recover from anesthesia was noted. An attempt was made to determine whether the recovered animal behaved normally. If the animal recovered to a normally reactive and ambulatory state, it was watched for signs of onset of paralysis, stupor, coma or convulsions. In all fatal cases the postoperative duration of life was recorded.

*Postmortem Studies*—Postmortem studies were made in all cases. In acute experiments the animals without symptoms were usually killed at the end of twenty-four hours. In other experiments the animals were kept as long as four weeks to determine histopathologic changes during the healing of lesions. All quantitative data concerned with percentage volumes of cerebral damage, however, refer to acute experiments.

The condition of the scalp and the calvarium was noted. The bone over each lesion was inspected, and blocks of the calvarium were prepared for microscopic study.

The brain was removed by dissection, severed from the spinal cord at the foramen magnum and fixed for forty-eight to seventy-two hours in 4 per cent formaldehyde solution. After fixation, a transverse incision was made through the medulla at the posterior angle of the fourth ventricle.

The volume of the brain in cubic millimeters was then determined by calculations from data on the weight of the brain in air and the weight while the organ was immersed in distilled water.

The volume of each lesion of the brain was determined as follows. A template covering the surface area of the lesion was prepared and transferred to graph paper with transverse and vertical lines at intervals of 1 mm. The squares included within the outline of the template were counted. This gave the surface area of the lesion in square millimeters. The lesion was then bisected in a direction perpendicular to the maximum diameter of the lesion. The maximum depth of the lesion was then measured to an accuracy of 0.25 mm. The product of the surface area and the depth was then taken as a standard approximation of the volume.

The volume per cent of cerebral damage was calculated as the ratio between the volume of the lesion and the volume of the brain

Microscopic study was done so that correlations could be made between the magnitudes of the lesion as determined grossly and microscopically

#### TYPES OF POSTOPERATIVE CLINICAL COURSE OBSERVED

Five types of postoperative clinical course were recognized Type 1 consisted of rapid, asymptomatic recovery

Type 2 was characterized by delayed recovery from anesthesia and stupor persisting for less than two hours followed by complete asymptomatic recovery

Type 3 was characterized by a well defined sequence of signs and symptoms There was slight delay in recovery from anesthesia The recovery, however, seemed to be complete The animal became ambulatory and behaved normally for a period varying from two to ten hours Then there was onset of stupor, followed by coma, which was terminated within two hours by convulsions and death This clinical course was critical, prognostically, because all animals which recovered consciousness postoperatively and then after a period of normal behavior lapsed secondarily into a stuporous state died within twenty-five hours after the time of production of the cerebral lesion

Type 4, more rapidly progressive, was characterized by incomplete recovery from anesthesia with persistence of unconsciousness, terminated by convulsions and death, usually within two to six hours after production of the lesion

Type 5 was seldom observed The animals never regained normal behavior postoperatively They were sluggish and responded to stimuli by hopping slowly away They did not eat and tended to remain in semireclining postures until they were near death from dehydration and malnutrition

No well defined hemiparesis, paraplegia or other localizing neurologic sign was encountered in any animal

#### PATHOLOGIC OBSERVATIONS

Immediately after production of a lesion there was no gross or microscopic change in periosteum, bone or dura There were pericapillary hemorrhages in the leptomeninges and the brain These were more prominent in the brain and were invariably restricted to the site of the lesion Significant bleeding into the subarachnoid space beyond the sharp limits of the lesion was not encountered Hemostasis was spontaneous and efficient after the immediate postoperative period

Lesions studied during the first few hours after production showed slight gross changes in periosteum, bone and dura The subdural space within the limits of the lesion gradually became obliterated as fibrinous exudate bound the leptomeninges to the dura There was no consistent evidence of continuous oozing of blood into the subarachnoid space or the cerebral part of the lesion Edema, however, developed progressively It was mild in the periosteum, the dura and the leptomeninges It was prominent in the brain within and adjacent to the volume of damaged cerebral tissue

Lesions studied microscopically during the first few hours after production showed early necrosis of cells, vasodilatation, intravascular thrombi, edema, interstitial hemorrhage and cellular infiltration, the inflammatory cells being few These changes were mild in the periosteum and the dura They were more conspicuous in the bone marrow, perhaps because of the rapid disintegration of hemopoietic cells The continuity of collagenous, reticular and osseous intercellular structure was essentially retained These matrices were surprisingly resistant to

change The continuity of structure of the leptomeninges was also retained Vasodilatation, intravascular thrombi, perivascular hemorrhage and necrosis of neural structure were prominent in the brain Ganglion cells disappeared rapidly Endothelial cells, fibrocytes and microglia cells were more resistant and in some lesions seemed to persist as structurally unimpaired units Inflammatory cell infiltration was mild in all locations There was no suppuration or widespread liquefaction necrosis (fig 2)

Lesions whose evolution was studied over a period of several weeks showed periosteum and bone rapidly returning to normal The leptomeninges became firmly fixed by fibrous adhesions to the dura and the cerebrum within the limits of the boundary of the lesion The cerebral lesions contracted progressively, so that by the end of five weeks large, purplish red, acute lesions had been converted to small, yellow, puckered, glial scars enclosing soft masses of degenerated cerebral tissue and cysts

Lesions whose evolution was studied microscopically over a period of several weeks showed slowly progressive healing Sections of the calvarium presented



Fig 2—A low power photomicrograph of a sagittal section of a lesion in the parietal lobe of a rabbit's brain This lesion, which is of very small dimensions, is twelve hours old Note the sharp margins and the convex base of the lesion in the midzone of the cortical gray matter Within the limits of the lesion all nerve cells have disappeared, but there is no other significant disintegration of structure Perivascular hemorrhage and leukocytic infiltration are inconspicuous

a peculiar formation of osteoid tissue in relation to the residual framework of "nonviable" bone There was no sequestration or suppuration of bone Even the continuity of "nonviable" and "viable" bone seemed to persist The dura, thickened by fibrosis, became intimately fused with the leptomeninges, which in turn were firmly bound by fibrous adhesions to the surface of the healing cortical lesion The acute inflammatory and neuronal degenerative changes in the cerebral lesions subsided within the first few days There was no suppuration As the few infiltrating polymorphonuclear leukocytes disappeared, macrophages laden with lipid materials derived from disintegrating neurons became conspicuous The process of resorption, phagocytosis and gliosis was slow There was no regeneration of neurons All ganglion cells disappeared Reparative processes finally converted the volume of injured cerebral tissue into a lesion which closely resembled a healed infarct of the human brain

## DIMENSIONS OF THE CEREBRAL LESIONS

Each cerebral lesion was essentially cylindric in shape. The bases of the cylinder, one adjacent to the leptomeninges and the other at a deeper level in the brain, were slightly convex. (See figures 3 and 4.) The subleptomeningeal



Fig 3—Photographs showing the superior convex surfaces of brains of rabbits containing bilateral parietal lesions. The lesions are six to twelve hours old. The clinical course of these animals was type 3. Note the discrete character of the lesions, with vasodilatation, edema and hemorrhage restricted to the areas of injury.

base was always of slightly greater diameter than the intracerebral base. Each lesion, therefore, was more like a solid truncated cone. The smallest lesions were



about 5 mm in diameter and 1 mm in depth. The largest lesions were about 25 mm in diameter and 7 mm in depth. Most lesions were from 15 to 20 mm in diameter and 3 to 5 mm in depth.

The total volume per cent of the brain occupied by lesions ranged from 1.33 to 32.5 (see table). About half of the series had single unilateral lesions. The remainder had either one lesion in each hemisphere or occasionally two lesions in one hemisphere.

There were several sources of error in the measuring of lesions. There was no significant error in the technic of measuring the volume of the brain. The differences, however, between the volume as measured and the true normal volume may be considerable. Formaldehyde fixation decreases the volume of the brain. However, it seems probable that the standard time of fixation eliminated significance

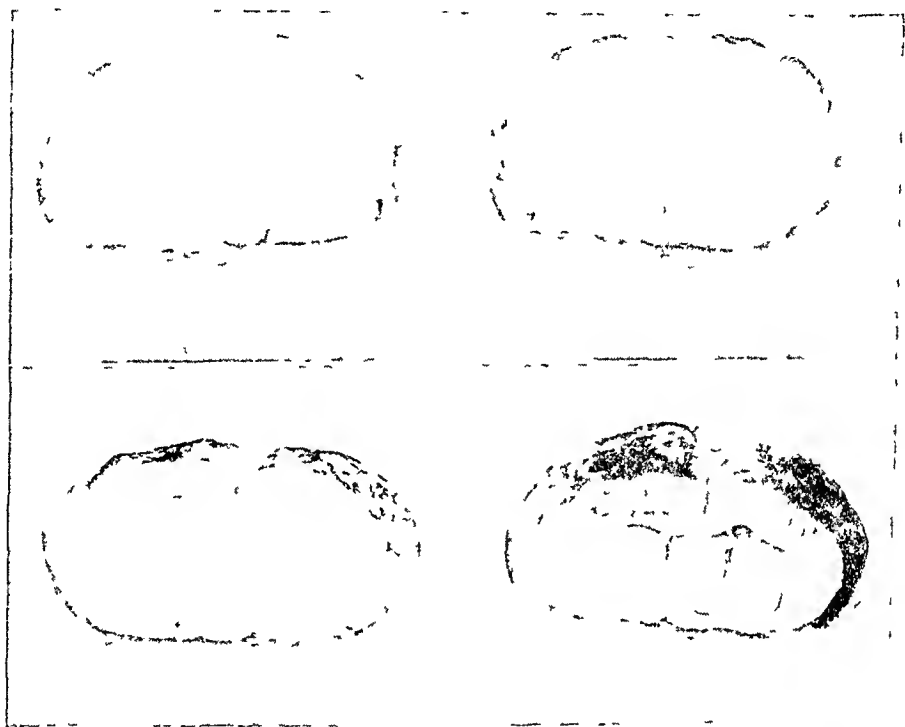


Fig. 4—Photographs showing sagittal sections of bilateral parietal lesions of brains of rabbits. The lesions are six to twenty-four hours old. They represent lesions which are of four different magnitudes in breadth, depth and volume. Each animal pursued a clinical course (type 1, 2, 3 or 4) in keeping with the illustrated differences of the percentage volume of cerebral damage. Note the distinct bases and margins of the lesions.

from this source of error. The lesion of the brain also changed the volume of the brain. When a lesion was acute, there was an increase in the total cerebral volume because of edema and hemorrhage within the lesion. These factors necessarily varied, increasing, in general, with increase of the volume of the lesion. When a lesion was old or healed, there was a decrease in total cerebral volume. Edema had subsided. Hemorrhage had resorbed. Degenerated cerebral tissue had disappeared, leaving a contracted scar. The over-all effect of old healing lesions was to minimize the actual volume per cent of cerebral damage.

*Relations Between Percentage Volume of Cerebral Damage, Type of Clinical Course and Duration of Life*

| Experiment No | Percentage<br>Volume<br>of Cerebral<br>Damage | Type<br>of Clinical<br>Course | Duration<br>of Life,<br>Hr * |
|---------------|---|-------------------------------|------------------------------|
| 16            | 13  | I                             |                              |
| 20            | 36  | I                             |                              |
| 2             | 37  | I                             |                              |
| 60            | 38  | I                             |                              |
| 18            | 38  | I                             |                              |
| 67            | 41  | I                             |                              |
| 62            | 41  | I                             |                              |
| 6             | 42  | II                            |                              |
| 62            | 42  | I                             |                              |
| 15            | 42  | I                             |                              |
| 51            | 43  | I                             |                              |
| 17            | 43  | I                             |                              |
| 21            | 43  | I                             |                              |
| 43            | 45  | I                             |                              |
| 112           | 45  | II                            |                              |
| 41            | 46  | II                            |                              |
| 70            | 47  | I                             |                              |
| 127           | 49  | I                             |                              |
| 36            | 50  | I                             |                              |
| 38            | 54  | I                             |                              |
| 69            | 55  | I                             |                              |
| 86            | 57  | II                            |                              |
| 85            | 60  | I                             |                              |
| 129           | 61  | I                             |                              |
| 111           | 61  | II                            |                              |
| 42            | 64  | II                            |                              |
| 83            | 68  | I                             |                              |
| 106           | 69  | I                             |                              |
| 61            | 70  | I                             |                              |
| 40            | 72  | I                             |                              |
| 116           | 76  | I                             |                              |
| 117           | 77  | I                             |                              |
| 3             | 78  | I                             |                              |
| 30            | 79  | I                             |                              |
| 36            | 79  | I                             |                              |
| 1             | 80  | I                             |                              |
| 22            | 80  | I                             |                              |
| 39            | 82  | I                             |                              |
| 61            | 84  | I                             |                              |
| 124           | 88  | I                             |                              |
| 84            | 89  | I                             |                              |
| 132           | 90  | I                             |                              |
| 14            | 91  | I                             |                              |
| 68            | 91  | I                             |                              |
| 9             | 93  | I                             |                              |
| 114           | 94  | I                             |                              |
| 44            | 94  | III                           | 7                            |
| 113           | 95  | III                           | 5                            |
| 76            | 96  | II                            |                              |
| 108           | 96  | I                             |                              |
| 10            | 101   | I                             |                              |
| 110           | 102   | I                             |                              |
| 25            | 103   | III                           | 7                            |
| 135           | 104   | I                             |                              |
| 59            | 111   | I                             |                              |
| 122           | 113   | I                             |                              |
| 107           | 114   | I                             |                              |

*Relations Between Percentage Volume of Cerebral Damage, Type of Clinical Course and Duration of Life—Continued*

| Experiment No | Percentage<br>Volume<br>of Cerebral<br>Damage | Type<br>of Clinical<br>Course | Duration<br>of Life,<br>Hr * |
|---------------|---|-------------------------------|------------------------------|
| 100           | 11.5  | IV                            | 3                            |
| 102           | 11.7  | I                             |                              |
| 57A           | 11.7  | I                             |                              |
| 137           | 11.9  | I                             |                              |
| 131           | 12.2  | III                           | 8                            |
| 75A           | 12.4  | I                             |                              |
| 133           | 12.6  | I                             |                              |
| 136           | 12.7  | I                             |                              |
| 149           | 12.8  | III                           | 20                           |
| 111           | 13.0  | V                             |                              |
| 154           | 13.5  | II                            |                              |
| 139           | 13.5  | IV                            | 5                            |
| 97            | 13.6  | III                           | 12                           |
| 19            | 13.6  | III                           | 5                            |
| 120           | 14.1  | I                             |                              |
| 140           | 14.3  | I                             |                              |
| 161           | 14.6  | IV                            | 3                            |
| 155           | 14.9  | V                             |                              |
| 98            | 15.0  | III                           | 24                           |
| 105           | 15.0  | I                             |                              |
| 123           | 15.2  | I                             |                              |
| 126           | 15.3  | III                           | 6                            |
| 138           | 15.7  | III                           | 6                            |
| 99            | 15.8  | III                           | 25                           |
| 81            | 15.9  | III                           | 2                            |
| 156           | 16.1  | III                           | 4                            |
| 158           | 16.2  | I                             |                              |
| 160           | 16.4  | III                           | 4                            |
| 121           | 16.4  | I                             |                              |
| 104           | 16.6  | II                            |                              |
| 141           | 16.9  | III                           | 3                            |
| 159           | 17.2  | V                             |                              |
| 90A           | 17.4  | II                            |                              |
| 128           | 17.5  | I                             |                              |
| 58A           | 17.7  | III                           | 11                           |
| 92A           | 17.8  | V                             |                              |
| 152           | 18.0  | III                           | 9                            |
| 125           | 18.0  | I                             |                              |
| 144           | 18.5  | III                           | 3                            |
| 157           | 18.7  | III                           | 4                            |
| 143           | 18.7  | III                           | 3                            |
| 153           | 19.1  | III                           | 3                            |
| 145           | 19.8  | IV                            | 2                            |
| 142           | 20.3  | III                           | 14                           |
| 151           | 21.3  | III                           | 5                            |
| 130           | 23.6  | III                           | 8                            |
| 7             | 32.5  | III                           | 4                            |

\* If no period of survival is stated, the animal did not die

It became necessary, therefore, to restrict critical comparison of one experiment with another to instances in which the lesions did not differ greatly in volume and age. In addition, uncontrolled variables played such an important part that we have attached no significance to data which fail to take into account a plus or a minus 10 per cent range of error in the actual determination of the volume per cent of cerebral damage.

RELATIONS BETWEEN CLINICAL COURSE AND PERCENTAGE  
VOLUME OF CEREBRAL DAMAGE

The clinical courses of animals following the production of acute lesions were with rare exceptions in accord with the five types which have been described. The type of clinical course could usually be correlated with a range of percentage volume of cerebral damage (table). The variable distribution of the damage within the extraventricular limits of the superior convexity of the cerebrum in the parieto-occipital regions was not important. Clinical findings in animals with large unilateral lesions were comparable with those in animals with smaller bilateral lesions leading to the same total percentage volume of damage.

All animals in which the range of damage lay between 1.33 and 9.43 volumes per hundred volumes of brain followed clinical courses of type 1 or type 2. They recovered normal behavior either promptly or after slightly prolonged recovery from the anesthesia. Thereafter they had no significant signs or symptoms. Of the 46 animals in this group, only 6 had even a prolonged recovery period (fifteen to thirty minutes) following anesthesia (type 2). The remainder behaved as if no injury had been produced (type 1).

Forty-nine animals with cerebral damage within the range 9.45 to 18.53 volumes per cent followed one of five types of clinical course. Twenty-one animals recovered promptly from the anesthesia and exhibited no symptoms thereafter (type 1). Four animals recovered slowly from the anesthesia and thereafter exhibited no symptoms (type 2). Seventeen animals, after slight delay in recovering from the anesthesia, returned to normal behavior but thereafter lapsed into stupor terminated by coma and death (type 3). The duration of normal behavior varied from one to about twenty hours, and the postoperative duration of life from two to about twenty-five hours. Three animals failed to regain full consciousness postoperatively. They remained stuporous, lapsed into coma and died within three to five hours after the operation (type 4). Four animals were in a postoperative stuporous condition for at least two days (type 5). It seemed that they would die of causes other than cerebral damage, so they were regarded as survivors and put to death. All animals of this group had a large amount of cerebral damage (13.05 to 17.8 volumes per cent).

All animals (9) with more than 18.53 volumes per cent of cerebral damage died within two to fourteen hours. Of these, 8 had a period of normal behavior before lapsing into secondary stupor and coma, terminated by death (type 3). The ninth animal remained unresponsive postoperatively and died in two hours (type 4).

The following conclusions concerning the effect of single unilateral or multiple simultaneous unilateral or bilateral closed cerebral lesions

were drawn First, if the cerebral damage does not exceed 9.43 volumes per cent, the animal survives without exhibiting significant signs or symptoms Second, if the cerebral damage is within the range 9.43 to 18.53 volumes per cent, the probability of survival is about one half, diminishing somewhat as the percentage volume of damage increases Third, when the cerebral damage is greater than 18.53 volumes per cent, no animal survives Fourth, the clinical counterpart of minimum lethal cerebral damage is the type 3 clinical course, namely, slight delay of postoperative recovery, return of normal behavior, and then lapse into stupor terminated by coma and death No animal recovered from the stupor which developed after a period of normal behavior

#### MEAN PERCENTAGE LETHAL VOLUME OF CEREBRAL DAMAGE

All animals with cerebral damage less than 9.45 volumes per cent survived, and all animals whose cerebral damage was 18.53 volumes per cent or more died (table) There were 49 animals with cerebral damage between 9.45 and 18.53 volumes per cent In this group, 29 survived and 20 died The average percentage volume of cerebral damage was 13.7 among the survivors and 14.9 among those which died The mean percentage volume of cerebral damage between the two groups was about 14.3 Therefore, about one half of a large group of animals with lesions of this magnitude might be expected to survive If the volume of lesions is greater than about 18.5 per cent of the volume of the brain, none may be expected to survive These data indicate that the evaluation of the usefulness of methods for reducing the mortality rate due to lesions of this type can best be carried out by studies of large groups of animals whose percentage volume of cerebral damage is about 14.3 per cent of that of the brain or small groups of animals whose percentage volume of cerebral damage is more than 18.5 per cent of that of the brain

#### COMMENT

Heretofore, experimental studies of acutely expanding intracranial lesions have been conducted principally by traumatic methods These methods introduce variables of concussion, cranial fracture, diffuseness of injury or craniotomy with its numerous complications With the hypothermal method, described in this report, these variables have been eliminated Other variables, such as location, dimensions and character of lesion, have been only partly controlled There were limits as to location in which a large volume of injury could be conveniently produced The most suitable locations were beneath those parts of the skull which were most accessible and least irregular or convex For this reason, all lesions recorded here were produced beneath the superior planar or superior-lateral convex surface of the cranial vault

They extended in some cases as far forward as the anterior-superior convex surface of the frontal lobes and as far posteriorly as the tips of the occipital lobes. The lateral, inferior and medial aspects of the cerebral hemispheres were not selectively damaged. When these areas of the brain were approached, the technic was more difficult and volumes of lesions were less easily reproduced. Similar trouble was encountered in the production of large cerebellar lesions.

The location of lesions, topographically speaking, was not only limited from the point of view of controlled cortical inactivation. Discrete, deep central cerebral lesions could not be produced without inactivating all overlying structure up to the surface of the cerebral cortex.

The dimensions of lesions of certain types were reproduced with reasonable accuracy in successive experiments. When a standard method was adhered to, the range of error in the production of lesions of large diameter (about 25 mm), depth (about 5 mm) and volume (about 2,000 cu mm) or lesions of medium diameter (about 15 mm), depth (about 3 mm) and volume (about 500 cu mm) was no greater than plus or minus 10 per cent of the desired values. The greatest errors occurred when lesions of large or medium diameter (15 to 25 mm), small depth (0.5 mm) and very small volume (75 to 200 cu mm) were desired. In other words, the range of error in the reproduction of a lesion increased with increasing divergence of values for diameter and depth.

The character of the acute lesions was not constant. There was a slight variation of the amount of hemorrhage within the volume of injured tissue (figs 3 and 4). Inasmuch as there was no hemorrhage beyond the limits of the lesion except when the lateral ventricular wall had been destroyed, this slight variation of bleeding within the boundaries of lesions seems to be unimportant. However, it cannot be disregarded, and doubtless suggests an explanation for the variability of clinical manifestations caused by comparable percentage volumes of cerebral damage in the same locations in different animals.

We are inclined, however, to regard variations of the amount of cerebral edema as more important than those of intracerebral hemorrhage. Edema was never conspicuous when hemorrhage was insignificant. It was always conspicuous in and adjacent to the most hemorrhagic lesions. It reached a maximum, grossly, some time after perivascular hemorrhage had stopped. We have concluded that progressive edema rather than progressive hemorrhage or progressive necrosis was the principal cause of the late onset of severe clinical signs and symptoms after several hours of normal postoperative behavior.

These experiments have established criteria which should be useful in evaluating methods of treatment. A survey of the postoperative clinical courses indicates that with rare exceptions recovery of con-

sciousness and apparent normal behavior over a period of twelve to fourteen hours were followed by permanent asymptomatic recovery. This clinical course was characteristic of cases in which the cerebral damage was less than 9.43 volumes per cent. A similar clinical course was observed in more than one half of the cases in which the cerebral damage was greater than 9.43 and less than 18.5 volumes per cent. Among the other cases with damage in this range and cases with damage in excess of 18.5 volumes per cent there were many in which the postoperative period of normal behavior was characterized by a late onset of stupor, coma and convulsions, terminated invariably by death. The average postoperative duration of life in this group was seven and a half hours. A smaller number of animals failed to recover from the anesthesia and persisted in a stuporous, semicomatose condition, which occasionally was prolonged (type 5) but which usually was terminated by death in two or three hours (type 4). All animals with these symptoms had lesions in excess of 11 volumes per cent.

It may be concluded, therefore, that the problem of rational treatment of acutely expanding closed cerebral lesions that are characterized principally by intracerebral edema and hemorrhage can now be approached from a quantitative standpoint. A method of producing closed lesions of desired dimensions and character has become available. The relations between signs and symptoms and the volume per cent of the cerebrum occupied by the lesion, or lesions, have been established. All animals which either lapsed secondarily into stupor following a postoperative period of normal behavior or had more than 18.5 volumes of cerebral damage per hundred volumes of brain failed to recover. Any therapeutic method which appreciably reduces the mortality rates in either of these groups of cases may be regarded as beneficial, and the degree of benefit may be subjected to quantitative scrutiny.

#### SUMMARY

Acute closed cerebral lesions characterized by hemorrhage, necrosis and progressive edema were produced hypothermally without interrupting the continuity of the calvarium or introducing the variables incidental to mechanical trauma. The dimensions and the locations of the lesions were controlled so that they could be reproduced topographically and quantitatively in successive animals. Although hemorrhage, edema and necrosis varied slightly in lesions which were otherwise identical, the variations were restricted to discrete volumes of injury.

When unilateral or bilateral lesions of the cerebrum occupied less than 9.4 volumes per hundred volumes of the brain, symptoms were negligible. When the lesions occupied 9.4 to 18.5 volumes per cent severe symptoms developed in many animals. The data indicated that

the minimum lethal volume of cerebral damage in 50 per cent of animals was 14.3 per cent of the volume of the brain.

Severe clinical courses with an average postoperative duration of about seven hours and fatal termination always occurred when the lesions occupied more than 18.5 volumes per cent of the brain. The great majority of animals that died had a postoperative period of normal behavior. Secondary lapse into stupor was a clear indication that coma impended and that death would occur within about twenty-four hours from the time of completion of the operation.

These data indicate that a quantitative experimental approach to the problem of the treatment of acutely expanding closed intracerebral lesions characterized by local necrosis, hemorrhage and edema can be made. The postoperative duration of life can be predicted from the magnitude of the lesions which are produced, and this duration is sufficient to permit evaluation of most therapeutic methods.



# EFFECT OF HEAT ON THE NUTRITIVE VALUE OF LACTALBUMIN

Chemical and Morphologic Changes

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AND

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DETROIT

THE EFFECT of heat on the nutritive value of proteins has long been a problem of considerable interest. For example, evidence has been presented that diminished growth observed during administration of heat-treated diets may be the result of damage to lysine<sup>1</sup>. Others have shown that in such experiments there is diminished "in vitro" enzymatic digestion of protein<sup>2</sup>. In view of the number of factors concerned it seemed desirable to carry out a concomitant chemical and morphologic study designed to evaluate more thoroughly the total situation. The material presented here represents part of the data obtained from experiments in which the effect of heat on the nutritive value of lactalbumin was studied, other data have been reported elsewhere<sup>3</sup>.

## EXPERIMENTAL PROCEDURE

The animals used were male albino rats approximately 25 days old and weighing between 40 and 60 Gm. The diets contained lactalbumin dry heated at 200 C for one hour (designated 200DL), lactalbumin dry heated at 140 C for one hour (designated 140DL), unheated lactalbumin (designated UNL), and lactalbumin autoclaved at 120 C for one hour under 15 pounds (6.5 Kg) of pressure (designated 120AL). In these diets the only variable was the treatment to which the protein had been subjected. A diet containing no protein but with an isocaloric dextrin replacement of protein was also used. These diets were balanced in all other respects<sup>3</sup>. The rats were divided into three groups.

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From the Departments of Anatomy and Physiological Chemistry, Wayne University College of Medicine

1 Greaves, E O, Morgan, A F, and Loveen, M K. *J Nutrition* **16** 115, 1938

2 Seegers, W H, and Matill, H A. *J Nutrition* **10** 271, 1935. Evans, R J, McGinnis, J, and St. John, J L. *ibid* **33** 661, 1947

3 Davis, R M, Rizzo, P, and Smith, A H. *J Nutrition* **37** 115, 1949. See also Davis, R. M. *The Effect of Heat on the Nutritive Value of Proteins and Amino Acids*, Detroit, Wayne University, 1948

Group 1 The animals of this group were paired and the pairs fed diet 200DL, 120AL and 140DL for three weeks and diet UNL for four weeks. The amount given was determined by the daily intake of the 200DL animals.

Group 2 These animals were allowed to eat diets 120AL, 140DL and UNL ad libitum for eight weeks.

Group 3 The "no protein" group was fed ad libitum for a three week period and compared with the 200DL animals.

Five rats were selected, wherever possible, from each dietary group and studied in detail. No attempt was made to study pair-fed animals (tables 1 and 2).

During the experimental period, body weights were determined weekly, slit lamp examinations for corneal vascularization were carried out biweekly, starting usually two weeks after the experiment was begun, and representative animals

TABLE 1—Diets (Protein—Lactalbumin)

|            | Diet | Degrees C | Type of Heat | Time |
|------------|------|-----------|--------------|------|
| 200DL      |      | 200       | Dry          | 1 hr |
| 140DL      |      | 140       | Dry          | 1 hr |
| 120AL      |      | 120       | Autoclave    | 1 hr |
| UNL        |      | 0         | 0            | 0    |
| No protein |      | 0         | 0            | 0    |

TABLE 2—Groups and Their Feeding

| Group  | Diets      | Rats | Duration, Wk | Type of Feeding   |
|--------|------------|------|--------------|-------------------|
| 1      | 200DL      | 5    | 3            | Ad libitum        |
|        | 120AL      | 5    | 3            | Pair fed to 200DL |
|        | 140DL      | 5    | 3            | Pair fed to 200DL |
|        | UNL        | 5    | 4            | Pair fed to 200DL |
| 2      | 120AL      | 4    | 8            | Ad libitum        |
|        | 140DL      | 5    | 8            | Ad libitum        |
|        | UNL        | 5    | 8            | Ad libitum        |
| 3      | No protein | 5    | 3            | Ad libitum        |
| Repent | 200DL      | 4    | 3            | Ad libitum        |

were anesthetized with pentobarbital sodium, given intraperitoneally, and roentgenograms were taken with the animal in the dorsoventral position. At the end of the experimental period tail blood was taken for determinations of hemoglobin. The animals were then killed either by decapitation or by administration of pentobarbital sodium. When concentrations of plasma proteins were to be determined, the blood of decapitated animals was drained into an evaporating dish containing sodium oxalate crystals. Complete autopsies were performed, and weights of organs were determined. Tissues and organs were fixed in Bouin's fluid in all cases, and in the case of group 2, in both Bouin's fluid and formaldehyde-Zenker solution. In this group a portion of the liver was also fixed in 4 per cent neutral formaldehyde. Most of the liver was placed in alcoholic potassium hydroxide for determinations of lipid. Eyes were fixed in various solutions: Carnoy-Lebrun solution, Bouin's fluid and 4 per cent formaldehyde. Eyes and skin were infiltrated with,

and embedded in, celloidin. Lower extremities were decalcified after Bouin fluid fixation, embedded in paraffin and sectioned sagittally. All other tissues were embedded in paraffin, sectioned at approximately 6 microns and stained with hematoxylin and eosin. In addition, frozen sections of formaldehyde-fixed liver were stained for lipids with scarlet red. In selected cases of corneal vascularization india ink was injected.

### RESULTS

*Health (group 1)*—The 200DL rats showed a notable decrease in appetite and activity. Their hair became scanty. They did not maintain body cleanliness and tended to become infested with lice. They appeared humpbacked, their general condition deteriorated steadily, and they usually died in three to four weeks. Because of this, the duration of experiments in group 1 was three weeks. The course of events for those rats fed the "no protein" diet was similar to that of the 200DL group. The 120AL group was the next most affected, they appeared to be chronically malnourished. Some of these rats gradually failed and eventually died. The 140DL and UNL rats were undernourished and stunted in growth.

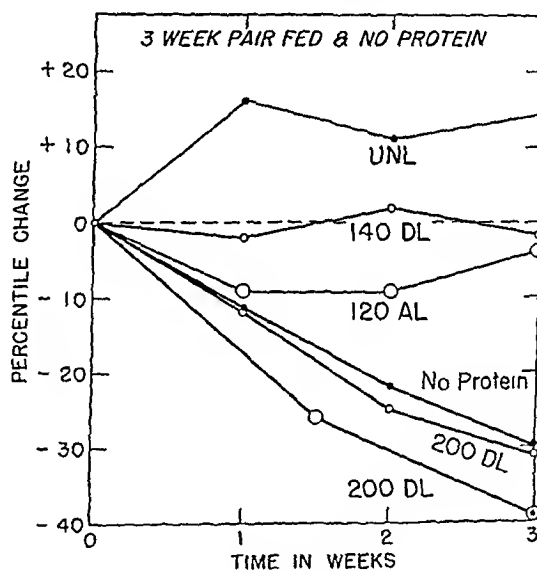


Chart 1—Group 1 and "no protein" body weights expressed as percentile changes from initial means

but remained active and maintained cleanliness. In group 2 the rats showed similar gradations in growth and well-being. Those in 120AL were most stunted, the 140DL group was intermediate, those in the UNL group grew at a normal rate.

*Weight*—As shown by the curves in charts 1 and 2, the losses of body weight were similar for rats in the 200DL and the "no protein" groups. Those in the 120AL, 140DL and UNL groups showed the same pattern of growth curves in both three and eight week periods, which indicated a consistently decreasing degree of malnutrition. Differences between groups were statistically significant.

*Organ-Body Weight Ratios*—Percentile weights of brain, lungs, heart, spleen, pituitary gland, adrenal glands, kidneys, testes and liver were calculated and subjected to statistical analyses. Thymus was not included because its location and separation from fat, blood vessels and lymph nodes could not be carried out.

with any reasonable degree of certainty. In the three week pair-fed group the figures were comparable for all organs except heart and kidney. The latter organs showed percentile values which were highest in the 200DL group and decreased progressively in the 120AL, 140DL and UNL groups. These differences were statistically significant. Testes and spleen showed such marked variability within groups that differences between groups were not significant. In the 120AL, 140DL and UNL ad libitum groups the organs may be classified as follows:

Those which showed inverse variation with body weight, i. e., 120AL animals which had the lowest body weights showed the highest percentile organ weights, 140DL animals were intermediate in both body and organ weights, and UNL animals with the highest body weights showed the lowest percentile organ weights. In most cases differences between group means of percentile weights for the same organ were statistically significant. These organs were brain, lungs, heart, adrenal glands and kidneys.

One organ which showed direct variation with body weight—testis.

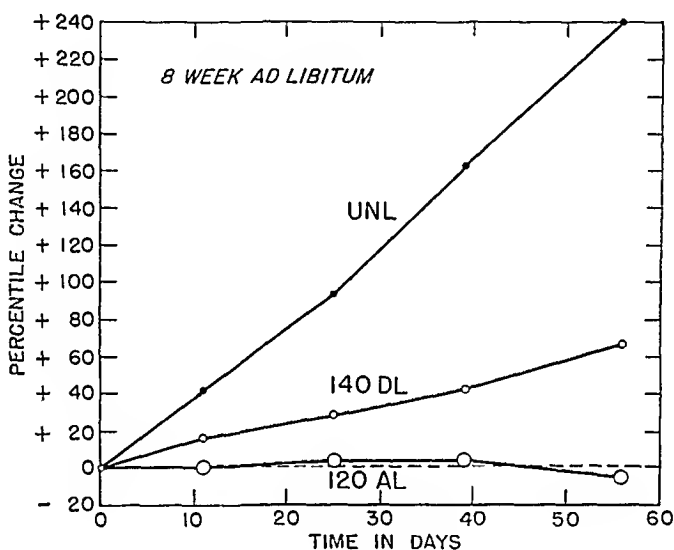


Chart 2—Group 2 body weights expressed as percentile changes from initial means

Others. The livers of 140DL animals showed the highest per cent of body weight and those of UNL the lowest. There was, however, no statistically significant difference between 120AL and 140DL.

These findings suggest that the weight of the liver may vary inversely with that of the body. The pituitary gland showed significant differences between all groups, but the error in measuring such small quantities was so large that the results were not considered meaningful. Nevertheless, 120AL presents the highest per cent of body weight, 140DL the lowest and UNL an intermediate per cent.

*Roentgen Examination*—Roentgen examination of controls and their diet mates in the three week pair-feeding experiment showed marked obliteration of epiphyseal lines in the 200DL group and little or no differences between those of the 140DL, 120AL and UNL (control) rats. The most prominent roentgen feature of the deficient eight week ad libitum rats was the generalized lack of skeletal growth.

The epiphyses of these animals were graded in width, the UNL being widest, 140DL intermediate and 120AL narrowest. No changes of degree of calcification were noted in either experiment.

**Hemoglobin**—Hemoglobin was determined on tail blood by the acid hematin method (table 3). As shown in table 3, the three week 120AL and 200DL groups showed probable anemia, while UNL and 140DL had relatively normal values. The degree of anemia in the "no protein" group was not significantly different from that in the 200DL. There were similar findings in the eight week group.

TABLE 3—*Hemoglobin*

| Study           | Diet         | Rats | Gm of Hemoglobin per 100 Cc of Blood | Standard Deviation | Standard Error |
|-----------------|--------------|------|--------------------------------------|--------------------|----------------|
| 3 wk pair fed   | UNL*         | 5    | 12.25                                | 0.85               | 0.43           |
|                 | 120AL        | 3    | 10.18                                | 1.63               | 0.94           |
|                 | 140DL        | 5    | 12.20                                | 0.14               | 0.06           |
|                 | 200DL        | 5    | 11.32                                | 0.71               | 0.32           |
|                 | No protein † | 5    | 10.24                                | 1.24               | 0.55           |
| 8 wk ad libitum | 120AL        | 4    | 10.36                                | 1.68               | 0.84           |
|                 | 140DL        | 5    | 11.96                                | 1.29               | 0.58           |
|                 | UNL          | 5    | 11.99                                | 0.50               | 0.22           |

\* This group was fed for four weeks.

† This group was included for comparison with the 200DL rats.

TABLE 4—*Plasma Protein*

| Study         | Diet  | Rats | Gm of Protein per 100 Cc of Blood | Standard Deviation | Standard Error |
|---------------|-------|------|-----------------------------------|--------------------|----------------|
| 3 wk pair fed | UNL*  | 4    | 5.09                              | 0.14               | 0.07           |
|               | 140DL | 5    | 5.95                              | 0.68               | 0.30           |
|               | 120AL | 5    | 5.44                              | 0.78               | 0.35           |
|               | 200DL | 5    | 3.43                              | 0.68               | 0.30           |

\* This group was fed for four weeks.

**Plasma Proteins**—Plasma proteins were determined by the micro-Kjeldahl technic with the aid of a Pregl distillation apparatus<sup>4</sup> (table 4). The low value for the 200DL group is the only significant observation.

**Liver Lipids**—The gravimetric method of Leathes and Raper<sup>5</sup> was used for determining the lipid content of liver. Since complete data were not available for the lipid contents of the liver of animals used in all other parts of this study, the material presented in table 5 is the complete study performed on liver lipids.

Normal liver lipid content varies greatly in different groups of animals,<sup>6</sup> therefore, the data are given for rats of the same litters that were used for

4 Pregl, F. Die quantitative organische Mikroanalyse, ed 2, Berlin, Julius Springer, 1912.

5 Leathes, J. B., and Raper, H. S. The Fats, ed 2, New York, Longmans, Green & Co., 1925.

6 Singal, S. A., and Eckstein, H. C. J. Biol. Chem. 140: 27, 1941.

experimentation at the same time. The absolute values are quite different in the two series but the increase in liver lipid content with increase in heat treatment is consistent and may be significant.

*Morphologic Aspects*—No deviations from the controls of any experimental group were noted in the following organs: heart, lungs, kidneys, ureter, bladder, prostate, seminal vesicles, ductus deferens, muscle, brain, pituitary gland, thyroid gland, parathyroid gland, adrenal glands and skin. Changes were noted in the following organs:

**Spleen** Hypoplasia of malpighian corpuscles was frequently noted in all groups.

**Bone** In the epiphyses, the width of the zone of proliferating cartilage was decreased, there was also diminution of the number of hypertrophic cells and of the size of the zone of invading capillaries, and there was formation of little or no provisional bone. These changes were not seen in either the three or the eight week UNL group. They were observed in only 2 140DL animals (one was an eight week ad libitum animal showing an unusual degree of stunting, and the other a three week pair-fed animal) and in only 1 120AL three week pair-fed animal. The same alterations were seen in 7 of 9 200DL animals and 3 of 3 "no protein" animals. No changes were observed in articular cartilages of any member of any series.

**Marrow** Decreased cellularity was evident in the femoral and tibial marrow of certain deficient rats. (No counts were done.) This was noted in 9 of 9 200DL rats, in 1 of 3 "no protein" rats and in the same 2 140DL rats which showed thinning of the epiphyses. Comparison of hemoglobin levels with the hypoplastic marrow showed no apparent relationship between the two. This may possibly be the result of hemoconcentration.<sup>7</sup>

**Thymus** When the thymus was found in the more deficient animals (200DL and "no protein"), marked atrophy of both medullary and cortical elements was noted. Lymph nodes were occasionally atrophic, but the change was not impressive. No group comparisons could be made.

**Testes** Deficient animals showed decreased spermatogenesis, which appeared to stop at the stage of primary and secondary spermatocytes. In only 2 140DL animals of the three week pair-fed groups did mature sperms develop. None of the animals of the "no protein" group showed complete spermatogenesis. In the eight week ad libitum group all UNL rats showed mature sperms, as did 2 of 5 140DL animals and 1 of 4 120AL rats.

**Gastrointestinal Tract** No changes were noted in the intestines of any animal in any group. Ulcers of the stomach were a significant finding in deficient animals. No studies are available for the three week pair-fed groups. In subsequent studies, ulcers were found at autopsy in 20 per cent of 200DL, 25 per cent of "no protein," 100 per cent of 140DL, 100 per cent of 120AL and 20 per cent of UNL rats. They were located in the stratified squamous epithelium of the prostomach and usually at its junction with the secretory portion. When few they were arranged about the esophageal orifice, when multiple they covered the prostomach. Histologically, each was an erosion in the stratified squamous epithelium with hypertrophy of the surrounding epithelial cells. There was usually leukocytic infiltration of the submucosa and the muscular layers below the ulcer.

<sup>7</sup> Metcoff, J., Favour, C. B., and Stare, F. J. *J. Clin. Investigation* **24**: 82, 1945.

**Eyes** They were examined with the slit lamp routinely. Corneal vascularization was found, usually beginning temporally, about two and one-half to three weeks after the animals were restricted to certain of the deficient diets. The vessels in all cases invaded a previously clouded cornea, although many clouded corneas were seen without vascularization. Some corneas appeared to be ulcerated, but fluorescein was not used to investigate this phenomenon further. Owing to the extreme pallor of some of the animals, the blood vessels were difficult to see. Vascularization was seen in 7 of 9 200DL rats and 2 of 5 "no protein" rats, and in no other group. It was impossible to confirm vascularization in all instances by histologic study, but this does not invalidate the findings with the slit lamp. Since the vessels grow out in a comparatively narrow spray, it is difficult to locate them unless serial sections are made. When vascularization was apparent, capillaries were actually growing into the substantia propria of the cornea. There was also some degree of leukocytic infiltration below the corneal epithelium. No other changes were noted except possibly edema of the substantia propria of the cornea in some instances. Cataract formation was not observed in any animal.

TABLE 5—*Liver Lipids*

| Study      | Diet   | Rats | Time on Diet, Wk | Gm of Lipid per 100 Gm of Liver (Mean) | Standard Deviation | Standard Error |
|------------|--------|------|------------------|--|--------------------|----------------|
| Pair fed   | UNL    | 5    | 4                | 13.73                                  | 2.64               | 1.18           |
|            | 140DL  | 5    | 3                | 14.97                                  | 3.01               | 1.30           |
|            | 120AL  | 5    | 3                | 17.73                                  | 7.50               | 3.35           |
|            | 200DL* | 3    | 3                | 21.80                                  | 5.96               | 3.44           |
| Ad libitum | UNL    | 5    | 8                | 6.15                                   | 1.07               | 0.45           |
|            | 140DL  | 10   | 8                | 7.68                                   | 1.17               | 0.37           |
|            | 120AL  | 3    | 5                | 8.44                                   | 0.73               | 0.42           |
|            | 200DL  | 3    | 3                | 10.57                                  | 1.77               | 1.02           |

\* The livers were removed from the animals six to twelve hours after death.

**Liver** Scarlet red preparations of frozen sections of formaldehyde-fixed livers showed the greatest amount of fat in the 120AL and the least in the 200DL and "no protein" groups. These findings were roughly confirmed by the hematoxylin-eosin sections, but they do not agree with those obtained by chemical determinations of lipid. As ascertained by chemical means, 200DL had the highest lipid content, histologically, their lipid content was the lowest. This suggests that the fat in the liver of the 200DL animal is deposited in a form differing from that of the fat observed in the other groups. The histologic distribution of fat was variable. In only 1 animal (a 120AL not presented in this series) of the 79 examined for liver fat was damage noted.

**Pancreas** Pancreatic necrosis was observed in 200DL, 120AL ad libitum and 140DL ad libitum animals. The most severe damage was in the 120AL ad libitum group. It appeared to be confined to the acini and consisted of vacuolation of cytoplasm leading eventually to a loss of cell boundaries and karyolysis and pyknosis of nuclei. In the 140DL ad libitum group early changes were seen in small focal areas, and in the 200DL group diffuse early changes were noted.

## COMMENT

It was hoped when the study was begun to establish a chemical and morphologic syndrome which could be associated with the feeding of heated protein. As was reported previously,<sup>3</sup> there is diminished digestion of heated lactalbumin. This study adequately supports the hypothesis of decreased digestibility, since findings quite similar to those presented here have been reported by others who have studied protein deprivation. The comparison of 200DL and "no protein" groups shows that the two are similar in general status, loss of weight, chemical changes and morphologic changes. This is further evidence in support of the hypothesis of the indigestibility of heat-treated lactalbumin. The 200DL and "no protein" groups are examples of acute protein deprivation and the 120AL and 140DL groups show its chronic effects. The findings in 120AL and 140DL pair-fed groups are further accentuated and complicated by the pair-feeding phenomenon, which adds partial starvation to the picture. Whether it is possible to separate the findings due to protein deprivation from those due to underfeeding alone is yet another problem.

*Organ-Body Weight Ratios*—In examining organ-body weight ratios in the three week pair-fed experiment we observed that the only differences between UNL, 120AL, 140DL and 200DL groups are in the kidneys and the hearts, both of which show a high percentile weight in 200DL animals and then decreasing values through 120AL, 140DL and UNL rats. It is a well substantiated phenomenon that kidneys are resistant to loss of weight<sup>8</sup>. The values for the heart, in this case, must be explained on the same basis. The spleen and the testes are so variable, in this study and those of others<sup>9</sup> that the few observations presented here cannot be significant in determining differences between dietary groups.

In explaining the fact that the percentile weights of brain, lungs, heart, adrenal glands and kidneys vary inversely with body weight in the eight week ad libitum group one must consider three possibilities. First, the differences in percentile variation are due merely to graded amounts of unmeasured protoplasm. To produce, then, inverse variation the organs weighed must grow at a similar pace in all groups and present similar total values. On examination of crude weights there is seen a gradation of group organ weights similar to that of

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8 Jackson, C. M. *The Effects of Inanition and Malnutrition upon Growth and Structure*, Philadelphia, P. Blakiston's Son & Co., 1925.

9 Webster S. H., Liljegren, E. J., and Zimmer D. J. *Am J Anat* **81** 477 1947.



body weights. Therefore, the difference is not due solely to diminished growth of unmeasured elements. Second, these organs may be considered those most essential to maintenance of body integrity and therefore those which take precedence in utilizing the available protein. A third hypothesis, one which is difficult to separate from the second, is that the organs in question have genetically determined growth preferences. The truth is undoubtedly a composite of the factors considered. The reverse variation of the testes, which increase in weight directly as body weight, indicates their superfluous function under conditions of strain. The liver is variable in response, the present findings suggest inverse variation.

*Hemoglobin, Plasma Proteins*—The relationship of hemoglobin and plasma proteins to protein intake has been studied in detail.<sup>10</sup> It has been shown that the concentration of plasma proteins and that of hemoglobin decrease with diminished intake of protein and that hemoglobin is retained in preference to plasma proteins.<sup>11</sup> The loss of plasma protein is primarily due to loss of the albumin fraction.<sup>10,11</sup> A tendency toward anemia was noted in the 200DL and 120AL groups and diminished plasma proteins in the 200DL group. From examination of the data from the 200DL group it is evident that the percentile loss of total plasma proteins is greater than that of hemoglobin. In the 120AL animals a low level of hemoglobin is present with a normal level of plasma proteins. These findings are difficult to explain. Since hemoconcentration is a feature of protein deprivation, early anemia in the 140DL group might possibly have been discovered if blood volumes had been studied.

*Liver*—Hepatic lipids have been shown to increase with the degree of protein deficiency and to exist probably in altered form when protein depletion is most severe. Weast, Groody and Morgan<sup>12</sup> described increased liver lipids in dogs fed heated casein.

*Testes, Thymus, Spleen*—The histologic changes observed in these organs are common phenomena. Severe atrophy of the thymus is a well recognized entity with various amino acid deficiencies<sup>13</sup> and also with inanition.

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10 (a) Zeldis, L. J., Alling, E. L., McCoord, A. B. and Kulka, J. P. *J. Exper. Med.* **82** 157, 1945. (b) Bieler, M. M., Ecker, E. E., and Spies, T. D. *J. Lab. & Clin. Med.* **32** 130, 1947. Metcalf and others.<sup>7</sup>

11 Whipple, G. H. *Am. J. M. Sc.* **203** 477, 1942.

12 Weast, E. O., Groody, M., and Morgan, A. F. *Am. J. Physiol.* **152** 286, 1948.

13 Maun, M., Cahill, W. M., and Davis, R. M. (a) *Arch. Path.* **39** 294, 1945. (b) **40** 173, 1945, (c) **41** 25, 1946.

*Ulcers*—Hoelzel and Da Costa<sup>14</sup> have shown the high incidence of rumenal ulcers with hypertrophied edges in animals fed low protein diets. Zucker, Berg and Zucker<sup>15</sup> described fundic hemorrhages, as well as rumenal ulcers, in animals fed such diets. These hemorrhages were not seen in our studies. Except for the acute deficiencies, the incidence of ulcers in our data bears a consistent relationship to the degree of dietary damage due to heat. Considering the peculiar structure of the rat's stomach, one infers that the corresponding lesion in human beings would probably be esophageal ulceration. Therefore, the transference of any information gained from studies of such ulcers in rats to human peptic ulcers must be made with definite reservations.

*Bone*—Saxton and Silberberg<sup>16</sup> described epiphyseal derangement and narrowing consequent to the underfeeding of adequate diets over a prolonged period. In the present series similar epiphyseal changes were found in the 200DL and "no protein" groups. No measurements or cell counts were made, so that slight differences may have been overlooked. Hypoplasia of marrow was also observed by Saxton and Silberberg.

*Corneal Vascularization*—Corneal vascularization may be produced in the rat by a variety of dietary deficiencies, such as avitaminoses, lack of certain essential amino acids, lack of certain minerals<sup>17</sup> and even by protein-free diets<sup>18</sup>. Tryptophane is one of the amino acids which has been studied extensively with respect to corneal vascularization<sup>19</sup>. The difference between the vascularization of tryptophane deficiency and that obtained by feeding 200DL and "no protein" diets is the presence in the latter of corneal clouding without cataract formation. This indicates that tryptophane deficiency was not the sole factor involved in this experiment. The involvement was intermediate to that obtained with leucine deficiency,<sup>13b</sup> which is quite severe, and that with histidine deficiency<sup>13c</sup>. The relative imbalances of nutritional elements brought about by the present study are not simple, so that undoubtedly there are

14 Hoelzel, F, and Da Costa, E. *Proc Soc Exper Biol & Med* **29** 382 1931

15 Zucker, T F, Berg, B N, and Zucker, L M. *J Nutrition* **30** 319, 1945

16 Saxton, J A Jr, and Silberberg, M. *Am J Anat* **81** 445, 1947

17 Dann, W J, and Darby, W J. *Physiol Rev* **25** 326, 1945. Seydenstricker V P, Schmidt, H L Jr, and Hall, W K. *Proc Soc Exper Biol & Med* **64** 59, 1947

18 Seydenstricker, V P, Hall, W K, Hock, C W, and Pund, E R. *Science* **103** 194, 1946

19 Totter, J R, and Day, P L. *J Nutrition* **24** 159, 1942. Albanese, A A, Randall, R McT, and Holt, L E Jr. *Science* **97** 312 1943. Albanese, A A and Buschke, W. *ibid* **95** 584, 1942. Albanese, A A. *ibid* **101** 619 1945

many hidden factors exerting their influences. It must be concluded that in the rat corneal vascularization is either a nonspecific mechanism due to the exaggerated sensitivity of its cornea to nutritional deficiencies or that the factors are multiple and/or complex and as yet not understood.

*Pancreatic Necrosis*—In this series the necrosis of the pancreas corresponds to that observed by Friedman and Friedman<sup>20</sup>. The physiologic significance of the findings was not investigated.

#### SUMMARY

Chemical and morphologic changes observed in rats in consequence of the feeding of heated lactalbumin are those of protein deprivation and inanition. For a constant temperature these changes are more impressive when lactalbumin is autoclaved than when the protein is dry heated.

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20 Friedman, S. M., and Friedman, C. L. *Canad. M. A. J.* **55**: 15, 1946.

## CARDIAC HYPERTROPHY

An Immediate Response to Starling's Law of Increased Energy Output of the Heart

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ACCORDING to Starling's law of the heart, the energy set free at each contraction of the heart is a simple function of the length of its muscle fibers. The increased energy necessary to expel a greater volume of blood per stroke or a normal volume against an elevated arterial pressure is brought about by lengthening of the fibers of the heart. This becomes even more pronounced in the diseased heart, in which there is progressive dilatation in an attempt to maintain a normal cardiac output. On the other hand, it is generally agreed that cardiac hypertrophy can occur only under conditions in which the muscle fibers are stretched or lengthened. How soon, therefore, does hypertrophy set in after the heart is exposed to such increased expenditures of energy? Is there a lag during which the so-called reserve power of the heart is utilized to meet the increased demands?

There are surprisingly few experimental data dealing with these questions. While there is a growing realization clinically that cardiac hypertrophy may manifest itself relatively early, particularly after such a condition with a known onset as traumatic arteriovenous aneurysm, there is ample experimental documentation that hypertrophy can occur within two, three or more weeks. There have been, however, only few studies in which cardiac hypertrophy was sought for and recognized in earlier stages. Stewart<sup>1</sup> found that cardiac hypertrophy was in progress at the end of five days in dogs on whom aortic valvulotomy had been performed. He found a distinct, though small, increase in water content of the heart in early stages of hypertrophy. Beznak and Hajdu<sup>2</sup> constricted the abdominal portion of the aorta just below the diaphragm in rats. They demonstrated hypertrophy of the heart in two days and found it to be well pronounced in a week. Heart weights alone were used as a criterion of hypertrophy without reference to body weight or

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From the Department of Pathology, University of Illinois College of Medicine

1 Stewart, H. A. *J. Exper. Med.* **13**: 187, 1911

2 Beznak, M. V., and Hajdu, I. *Schweiz. med. Wchnschr.* **76**: 390, 1946

other standards. In a more recent study these authors<sup>3</sup> narrowed the ascending aorta of the albino rat by means of a silver ring. They found that the percentage dry weight and ash content of the heart increased during the first days of the hypertrophy, declining later to a normal level.

The most thorough study to date along these lines is that by Hitchings, Daus and Wearn.<sup>4</sup> Weights and chemical analyses of rabbits' hearts were obtained after rupture of an aortic valvular leaflet at intervals from one to two hundred and fifty-seven days, with emphasis on the third day. Only 4 animals were studied on the first day, 3 on the second and 14 on the third day. They concluded that cardiac hypertrophy occurred within the first three days and that it was characterized within this period by an increase in the extracellular phase of a magnitude amounting to interstitial edema. To this, they ascribed, in great part, the increased weight of the heart. At the same time there was also demonstrable an increase in the intracellular phase. After the third day the extracellular phase was at no time in excess over that found in the normal heart while the intracellular phase increased progressively for several weeks.

It has been shown<sup>5</sup> that cardiac hypertrophy can regularly be obtained by feeding chicks a diet containing excess sodium chloride. It seemed particularly advantageous to test the speed of hypertrophy by this means. No operative procedures were necessary. Large numbers of animals could be used under strictly standardized conditions and, as opposed particularly to the experimental production of aortic insufficiency or stenosis, this procedure did not entail any possible abrupt interference with the coronary circulation. It had the disadvantage that an electrolyte with water-retaining properties was used. It was largely for that reason that a twenty-four hour period was chosen for intensive study as it was expected that, following a given period of exposure to salt, the induced flux in body water would have become stabilized.

#### METHODS

The birds employed in these experiments were female New Hampshires except in a few instances in which cockerels of the same breed were used. This will be indicated in its appropriate place in the text. The birds were received in the laboratory when they were a day old. They were kept in electrically heated brooders for twenty-one days and fed a commercial starting chow. They were then placed in individual wired cages. They were given a commercial growing chow ad libitum for periods of one to six days, varying with different groups, and for nine days in experiments lasting four, nine and fifteen to sixteen days, respec-

3 Hajdu, I., and Beznak, M. V. *Arch Biol Hungarica* **17** 213, 1947  
abstracted *Excerpta med* (Sect 5) **1** 284, 1948

4 Hitchings, G. H., Daus, M. A., and Wearn, J. T. *Am J Physiol* **138**  
527, 1943

5 Krakower, C. A., and Heino, H. E. *Arch Pathol* **44** 143 1947

tively Thereafter they were restricted to 23.5 Gm of food daily for periods varying from less than a day to eight days At the onset of the experiment control birds were given 23.5 Gm of food and the experimental animals 23.5 Gm of food plus 1.5 Gm of sodium chloride The food was set out at 9 a m, and a measured quantity of tap water was supplied All food and water were withdrawn at 6 p m of the same day for experiments of twenty-four hours' duration or the evening prior to the day on which the animals were to be killed for experiments of longer duration The same amount of food with or without sodium chloride was supplied daily to the birds in experiments lasting two and three days, but thereafter the daily regimen was changed in the following progressive fashion for controls, 23.5 Gm of food and 6.5 Gm of cellulflour® (a specially treated cellulose, used as roughage) followed by an increase of 5 Gm of cellulflour® every three days, for experimental animals, 23.5 Gm of food and 4.7 Gm of cellulflour® and 1.8 Gm of sodium chloride, followed by an increase of 4.7 Gm of cellulflour® and 0.3 Gm of sodium chloride every three days

In the groups of birds used for the determination of intracellular and extracellular phase in heart and muscle, a slightly different procedure was followed After removal from the brooders on the twenty-first day, they were fed the growing chow ad libitum for fourteen days Thereafter they were restricted to 30 Gm of food daily for eleven and thirteen days for subsequent experiments that were to last one and three days, respectively On the day the experiment was started, the control animals continued to receive 30 Gm of food while the experimental ones were given 28.2 Gm of food and 1.8 Gm of sodium chloride For the experimental period lasting sixteen days the same amounts of food or the same proportions of food and sodium chloride were given the birds daily, but this regimen was started immediately after the fourteen day period of food ad libitum Food and water were withdrawn at 6 p m on the day preceding the one on which the animals were to be killed

*Cardiac Weights*—The animals were weighed immediately prior to killing With the bird under light chloroform anesthesia the thorax was rapidly opened, and a large aortic branch was severed The bird was allowed to die by exsanguination The heart was rapidly removed, the aorta being severed at the level of origin of the large trunks in all instances The chambers of the heart were rapidly opened, and all surfaces were blotted with filter paper The heart was rapidly transferred to a previously weighed bottle After weighing, the heart was either minced first and then chilled or, in reverse, it was chilled first and then minced The minced hearts were transferred as completely as possible into previously weighed and chemically cleaned glass fiber bags These were reweighed and placed in desiccators over calcium chloride The desiccators were connected to a vacuum jet Constant dry weights were obtained within two weeks The glass fiber bags were then placed in a Soxhlet apparatus Fat was extracted with anhydrous ethyl ether continuously for eight hours in each of two days, a total of sixteen hours, but the bags and contents were left immersed in ether for the intervening and following nights They were then placed in a desiccator under vacuum overnight and subsequently weighed

*Carcass Weights*—When the carcass of the animal was saved, the following procedure was adopted With the bird under light chloroform anesthesia, the thorax was opened, and as much blood as possible was withdrawn from the heart by means of a syringe and needle The blood was transferred to a test tube and allowed to clot The heart was then removed and treated in the manner described in the foregoing paragraph Care was taken throughout the whole of the procedure to prevent loss of feathers and excessive evaporation from the exposed

portions of the carcass and to minimize loss of blood. The clot in the test tube and whatever clots were found in the heart were returned to the carcass. The latter was weighed, wrapped in wax paper and then stored in a cold room at 0 Centigrade. It was subsequently ground to a fine powder in a cast iron mortar with a porcelain pestle. Liquid air was used to keep the carcass solidly frozen during the process of grinding. On warming, the powdery material formed a mash which was thoroughly mixed in a previously weighed container. The whole mash was then weighed and, with precautions against evaporation being observed, aliquot samples were removed and placed in weighed glass fiber bags. After weighing, extractions of water and fat were performed in the same manner as with the hearts.

*Cardiac Nitrogen*—In determining nitrogen on the fat-free dried material of the heart and carcass a semimicro-Kjeldahl method was used with the modification made by Ma and Zuazaga<sup>6</sup> for amounts of material greater than that ordinarily allowed for strict micromethods. The distillation of ammonia was performed in Pregl's micro-Kjeldahl apparatus as modified by Goebel.<sup>7</sup>

*Cardiac Glycogen*—The glycogen content of the heart was determined by the method of Good, Kramer and Somogyi,<sup>8</sup> except that 1 normal sulfuric acid was used instead of 0.6 normal hydrochloric acid, as suggested by Sahyun.<sup>9</sup> The glucose of the digest was determined colorimetrically by Benedict's copper method.<sup>10</sup>

In preparing the heart for estimation of glycogen content, the animal was anesthetized with ether. The thorax was opened and the heart was rapidly removed. It was blotted and immediately immersed in postassium hydroxide.

*Intracellular and Extracellular Phase of Heart and Skeletal Muscle*—The birds were lightly anesthetized with ether. The thorax was rapidly opened. Blood was withdrawn from the heart and allowed to clot under oil. One of the large aortic branches was then severed to complete the exsanguination of the animal. The heart was rapidly removed. The aorta and the pulmonary arteries were dissected away, and all visible epicardial fat was trimmed. The chambers were opened, and all surfaces of the heart were blotted free of blood. The anterior and lateral muscles of the thigh were carefully trimmed of fat and fascia. Both heart and muscle were placed in weighing bottles, chilled and minced. The serum obtained from the clotted blood under oil was measured with a 1 cc volumetric pipet into weighing bottles which had been dried in the oven at 100 C for forty-eight hours.

The procedure of Eichelberger and Bibler<sup>11</sup> was followed in the drying and defatting of serum, heart and skeletal muscle. Serum and tissue chloride were determined in duplicate by the wet ashing method of Van Slyke.<sup>12</sup> For tissue chlorides the procedure was modified as suggested by Eichelberger<sup>13</sup> in the following manner. 100 mg of the powdered tissue was weighed on highly glazed paper and transferred quantitatively to a digestion tube, 5 cc of water and 1 cc of 0.075 normal silver nitrate were added. The mixture was allowed to stand

6 Ma, T S, and Zuazaga, G. *Indust & Engin Chem (Anal Ed)* **14** 280 1942

7 Peters, J P, and Van Slyke, D D. *Quantitative Clinical Chemistry Methods*, Baltimore, Williams & Wilkins Company, 1932

8 Good, C A, Kramer, H, and Somogyi, M. *J Biol Chem* **100** 485, 1933

9 Sahyun, M. *J Biol Chem* **93** 227, 1931

10 Benedict S R. *J Biol Chem* **92** 141, 1931

11 Eichelberger, L, and Bibler, W G. *J Biol Chem* **132** 645, 1940

12 Van Slyke, D D. *J Biol Chem* **58** 523, 1923

13 Eichelberger, L. Personal communication to the authors

overnight Four cubic centimeters of concentrated nitric acid was added and the material allowed to digest

All calculations were based on the equations outlined by Hastings and Eichelberger<sup>14</sup> except that the amount of detectable blood in heart or muscle was so small that for all intents and purposes it was negligible The results were therefore expressed on a fat-free basis

Potassium determinations were performed on fat-free dry heart and skeletal muscle by the method of Shohl and Bennett<sup>15</sup> Owing to the limited amount of fat-free dried cardiac material, it was necessary to pool some of the hearts in instances

#### OBSERVATIONS AND RESULTS

*Water and Sodium Chloride Intake in Twenty-Four Hour Experiments*—The animals were exposed to food and water for a nine hour period on the day prior to killing In practically all instances their intake of food had been restricted for several days The amount of food was just adequate to maintain a slow rate of growth, capable at this age of producing a maximum body weight of 500 to 600 Gm

TABLE 1—*Cardiac Weights and Ratios of One Day Experiment*

|  | Control               | Experimental          |               |
|--|-----------------------|-----------------------|---------------|
| Animals                                  | 32                    | 33                    |               |
| Body weight, Gm                          | 286.7 ± 25.7          | 288.5 ± 25.9          |               |
| Wet heart weight, Gm                     | 1.677 ± 0.159         | 1.828 ± 0.189         |               |
| Dry heart weight, Gm                     | 0.449 ± 0.054         | 0.477 ± 0.062         |               |
| Fat free dry heart weight, Gm            | 0.298 ± 0.029         | 0.315 ± 0.029         |               |
| Wet heart weight per cent                | 0.586 ± 0.049         | 0.636 ± 0.063         |               |
| Body weight                              | SE <sub>m</sub> 0.009 | SE <sub>m</sub> 0.011 | P < .01       |
| Fat free dry heart weight, per cent      | 17.468 ± 0.723        | 17.010 ± 0.885        |               |
| Wet heart weight                         | SE <sub>m</sub> 0.134 | SE <sub>m</sub> 0.154 | .05 > P > .02 |
| Total fat free solids of heart, per cent | 19.283 ± 0.789        | 18.732 ± 0.890        |               |
| Fat free wet weight of heart             | SE <sub>m</sub> 0.147 | SE <sub>m</sub> 0.162 | P < .01       |
| Total water of heart, per cent           | 80.717 ± 0.789        | 81.268 ± 0.890        |               |
| Fat free wet weight of heart             | SE <sub>m</sub> 0.147 | SE <sub>m</sub> 0.162 | P < .01       |

The values for P were obtained from Fisher's tables and were calculated from the standard error of the mean signified by SE<sub>m</sub> Values for P less than 0.05 are considered to be statistically significant

over a period of weeks The object of this was to obtain, as far as possible, animals of uniform weight As a result of such caloric restriction, most of the birds consumed the food given them within three to five hours On the assumption that the content of sodium chloride in the chick feed was 0.5 per cent, the control birds on the average consumed 0.4 Gm of sodium chloride per kilogram of body weight, while the experimental ones consumed 5.5 Gm per kilogram The consumption of water during the nine hours was 295 ± 150 cc per kilogram of body weight per bird for 32 control animals and 669 ± 119 cc per kilogram of body weight per bird for 33 experimental birds

*Cardiac Weights in Twenty-Four Hour Experiment*—Table 1 represents a composite of four sets of experiments performed at different times and with parallel results each time The average body weights of control and experimental animals were almost identical Despite this, it is evident that the heart weights of the experimental animals were appreciably heavier than the control weights, with a highly significant statistical difference in the wet heart weight-body weight ratios Numerically the ratio for the experimental birds was 8.4 per cent greater than the control ratio In addition it will be noted that both the dry heart weights

14 Hastings, A. B., and Eichelberger, L. J. Biol. Chem. **117**: 73, 1937

15 Shohl, A. T., and Bennett, H. B. J. Biol. Chem. **78**: 643, 1928



and, more significantly, the dry fat-free heart weights of the experimental animals were greater than the controls. It is clear, however, in view of the reduced percentage of fat-free solid substance of the experimental whole wet or fat-free wet hearts, as well as of the percentage of total water, that there was a statistically significant increase in the amount of water in these hearts. It was for this reason that water partition in heart and, for comparison, that of skeletal muscle were studied in accordance with the procedure of Hastings and Eichelberger<sup>14</sup>

*Intracellular and Extracellular Phase of Heart and Skeletal Muscle*—From table 2 it will be noted that in the first group of animals, on a one day experiment, the extracellular fluid of the hearts of the experimental animals exceeded that of the controls by 8.9 per cent. This is an appreciable increase. Nevertheless, if one compares it with the findings in skeletal muscle, one finds that there, too,

TABLE 2—*Extracellular and Intracellular Water of Heart and Skeletal Muscle*

| Group 1 One Day Experiment (6 Control and 6 Experimental Animals)                 |                   | H <sub>2</sub> O,<br>Gm per Kg * | ClmM,<br>per Kg † | Fel ‡        | (H.O)c §    |
|---|-------------------|----------------------------------|-------------------|--------------|-------------|
| Serum   | Control           | 957.7 ± 3.3                      | 115.7 ± 3.9       |              |             |
|   | Experimental      | 953.8 ± 3.8                      | 118.9 ± 4.2       |              |             |
| Skeletal muscle   | Control           | 794.4 ± 3.6                      | 7.4 ± 1.1         | 59.2 ± 9.0   | 782.8 ± 4.0 |
|   | Experimental      | 790.7 ± 8.9                      | 8.5 ± 1.2         | 68.6 ± 10.0  | 776.7 ± 8.4 |
| Heart   | Control           | 827.8 ± 5.2                      | 20.8 ± 2.5        | 165.5 ± 18.4 | 795.6 ± 7.5 |
|   | Experimental      | 829.9 ± 5.2                      | 23.3 ± 2.3        | 180.3 ± 21.8 | 794.5 ± 8.3 |
| Group 2 Control, 1 Day, 3 Day and 16 Day Experiments (8 Animals in Each Subgroup) |                   |                                  |                   |              |             |
| Serum   | Control           | 954.8 ± 3.1                      | 116.0 ± 1.8       |              |             |
|   | 1 day experiment  | 957.9 ± 1.4                      | 116.8 ± 5.2       |              |             |
|   | 3 day experiment  | 953.7 ± 4.5                      | 113.0 ± 1.0       |              |             |
|   | 16 day experiment | 954.7 ± 2.4                      | 114.3 ± 1.7       |              |             |
| Skeletal muscle   | Control           | 785.4 ± 3.5                      | 7.3 ± 0.6         | 58.5 ± 5.1   | 772.7 ± 2.8 |
|   | 1 day experiment  | 782.6 ± 4.1                      | 7.1 ± 1.0         | 59.2 ± 7.5   | 769.5 ± 4.4 |
|   | 3 day experiment  | 791.1 ± 2.7                      | 7.5 ± 1.2         | 61.8 ± 9.5   | 777.9 ± 3.7 |
|   | 16 day experiment | 790.1 ± 4.0                      | 7.3 ± 0.9         | 59.3 ± 7.3   | 777.5 ± 3.8 |
| Heart   | Control           | 834.5 ± 7.1                      | 25.4 ± 2.6        | 204.6 ± 23.5 | 794.4 ± 7.8 |
|   | 1 day experiment  | 838.5 ± 8.2                      | 25.6 ± 2.6        | 204.0 ± 19.4 | 799.2 ± 8.3 |
|   | 3 day experiment  | 836.8 ± 5.5                      | 24.4 ± 1.9        | 201.9 ± 15.3 | 797.9 ± 5.8 |
|   | 16 day experiment | 833.5 ± 5.8                      | 23.9 ± 3.5        | 195.2 ± 28.3 | 795.5 ± 5.8 |

\* The total water is expressed in grams per kilogram of fat-free material

† ClmM is chloride expressed in millimols per kilogram of fat free material

‡ Fel is the extracellular fluid expressed in grams per kilogram of fat free tissue

§ (H.O)c is intracellular water expressed in grams per kilogram of fat free intracellular phase

there was a marked increase in extracellular fluid in the experimental animals, amounting to 10.8 per cent. This finding in skeletal muscle, therefore, reflects the more generalized increase of fluid in the extracellular spaces of the body, in which the heart shared. At the same time it will be noted that the concentration of water in the intracellular phase decreased. Actually, as calculated, there was an absolute increase in the amount of intracellular water in the experimental hearts. The following figures will help to clarify this. The average values for the 6 control animals for body weight, wet heart weight, wet heart weight-body weight ratio and fat-free wet heart weight were 427.8 ± 35.4 Gm, 1.856 ± 0.247 Gm, 0.432 ± 0.029 and 1.781 ± 0.235 Gm, respectively. For the 6 experimental animals the values were 398.6 ± 39.8 Gm, 2.146 ± 0.243 Gm, 0.541 ± 0.07 and 2.087 ± 0.239 Gm, respectively. The total amount of intracellular water of the control hearts was on the average 1.182 Gm, with a fat-free solid substance of 0.306 Gm, and that of the experimental hearts was 1.357 Gm and 0.385 Gm, respectively, i.e., despite a smaller body mass, the experimental animals had hearts which on a heart weight-body weight ratio were 25 per cent heavier. Although

a good deal of this was due to increase of extracellular fluid, there was nevertheless almost an equal percentage increase in the total amount of intracellular water and a greater percentage increase in fat-free solid substance

That there was, however, variation in the amount of extracellular fluid of the heart in groups of animals aside from that in animals within each group is borne out by the findings in group 2. It will be noted here that there was no increase during the first day, while there was a progressive drop during the third and sixteenth days. The values for skeletal muscle likewise reflected the absence of edema. On the other hand, the intracellular concentration of water was increased over that of the controls in all three experimental periods. The average values for the control animals for body weight, wet heart weight, wet heart weight-body weight ratio, fat-free wet heart weight and total fat-free solids were  $449 \pm 49.8$  Gm,  $1806 \pm 0.268$  Gm,  $0.403 \pm 0.048$ ,  $1712 \pm 0.256$  Gm, and  $0.283$  Gm, respectively. For the one day experimental animals the values were  $431.2 \pm 18.2$  Gm,  $1858 \pm 0.144$  Gm,  $0.432 \pm 0.039$ ,  $1758 \pm 0.148$  Gm, and  $0.284$  Gm. The increase in wet heart-body weight ratio was 7.1 per cent, in keeping with the general average of the large group exemplified in table 1. While there was little increase in the fat-free solid substance of the hearts of the lighter experimental animals, this rose rapidly to  $0.309$  Gm on the third experimental day, when body weight was  $443 \pm 31.5$  Gm, wet heart weight  $1976 \pm 0.323$  Gm, fat-free wet heart weight  $1895 \pm 0.306$  Gm and wet heart weight-body weight ratio  $0.446 \pm 0.067$ , an increase of 10.7 per cent over the controls. On the sixteenth experimental day the fat-free solid substance had risen to  $0.364$  Gm in animals with body weight of  $466.2 \pm 37.1$  Gm, wet heart weight of  $2276 \pm 0.348$  Gm, fat-free wet heart weight of  $2187 \pm 0.347$  Gm and wet heart weight-body weight ratio of  $0.490 \pm 0.081$ , or an increase of 21.5 per cent over that of the controls.

The increase in solid substance of the experimental hearts was represented not only by an increase in nitrogen (see table 3) but by an increase in potassium as well. The values for the control one day, three day and sixteen day experimental hearts of group 2 were as follows: 76.06, 76.18, 77.41, and 79.11 miliequivalents (mEq) per kilogram of fat-free dry substance, respectively. While in the hearts, therefore, there was an increasing amount of potassium during the period of hypertrophy, skeletal muscle revealed no such increase, in fact, the values of skeletal potassium were at all times lower than the controls—as indicated by the following figures: 89.23, 85.03, 86.81, and 85.70 mEq per kilogram of fat-free dry substance for control, one day, three day and sixteen day experimental birds respectively.

In summary, it seems evident from these results that on the average there is a constant increase in intracellular water in the experimental hearts in early hypertrophy. This is to be regarded as an integral part of cardiac hypertrophy. The extracellular fluid phase, however, is far more labile and may be either more or less proportionate to the increased mass of the heart or, if greater than that, reflective rather of a disturbance in the total elimination of excessive fluid than of cardiac hypertrophy.

*Comparison of Heart and Carcass Weights in Twenty-Four Hour Experiment—* Changes in cardiac mass should be appropriately evaluated by reference to some standard. It would have been preferable to use the surface area of the body for such a standard but with the feathered animal this was not feasible. It was felt that if body weight was used as a point of reference the whole carcass should be treated in the same manner as the heart. In table 3 the relationship between heart and carcass values is represented for 7 control and 8 experimental animals. It will be noted that the same statistically significant increase of wet heart-whole

body weight ratio is maintained in the experimental animals as compared with the controls, whether the ratio is determined as dry heart weight dry carcass weight, fat-free dry heart weight fat-free dry carcass weight, or fat-free wet heart weight fat-free wet carcass weight. It is also true that with increase of fat-free solid substance of the experimental hearts there is increase of total nitrogen in these hearts and in the ratio of the total nitrogen of the heart to that of the carcass.

*Glycogen Content of Heart in Twenty-Four Hour Experiment*—There was considerable individual variation in the glycogen content of the heart. For 9 control animals, including 2 cockerels, there was on the average 109.7 mg of glycogen per hundred grams of wet heart, with a range of 46.4 to 218.8 mg, whereas for 10 experimental animals, including 2 cockerels, there was 110.7 mg of glycogen per hundred grams of wet heart, with a range of 59.2 to 195.0 mg. There was, therefore, on the whole, no appreciable increase in the glycogen content of the experimental hearts.

TABLE 3—*Comparison of Heart and Carcass Weights of One Day Experiment*

|                               | Wet Weight,<br>Gm                                       | Dry Weight,<br>Gm                                       | Fat Free<br>Dry Weight,<br>Gm                           | Fat Free<br>Wet Weight,<br>Gm                     | Total<br>Nitrogen,<br>Gm                                | Total Water<br>per 100 Gm of<br>Fat Free<br>Wet Tissue |
|-------------------------------|---|---|---|---|---|--|
| Control Animals—7             |   |   |   |   |   |  |
| Carcass                       | 291.1 ± 22.2  | 95.5 ± 8.9  | 76.0 ± 7.0  | 271.6 ± 22.4                                      | 9.24 ± 0.80   | 72.0 ± 0.4<br>SE <sub>m</sub> 0.2                      |
| Heart                         | 1.686 ± 0.074   | 0.462 ± 0.053   | 0.290 ± 0.012   | 1.514 ± 0.071                                     | 0.038 ± 0.002   | 80.826 ± 0.685<br>SE <sub>m</sub> 0.259                |
| Heart, per cent of<br>Carcass | 0.581 ± 0.038<br>SE <sub>m</sub> 0.014                  | 0.486 ± 0.059<br>SE <sub>m</sub> 0.022                  | 0.384 ± 0.037<br>SE <sub>m</sub> 0.014                  | 0.559 ± 0.035<br>SE <sub>m</sub> 0.013            | 0.414 ± 0.035<br>SE <sub>m</sub> 0.01                   |  |
| Experimental Animals—8        |   |   |   |   |   |  |
| Carcass                       | 291.9 ± 14.2  | 91.7 ± 8.0  | 76.2 ± 4.6  | 276.5 ± 14.7                                      | 9.10 ± 0.69   | 72.4 ± 1.7<br>SE <sub>m</sub> 0.6<br>6 > P > 5         |
| Heart                         | 1.917 ± 0.142   | 0.489 ± 0.054   | 0.330 ± 0.029   | 1.758 ± 0.143                                     | 0.045 ± 0.003   | 81.219 ± 0.871<br>SE <sub>m</sub> 0.308<br>4 > P > 3   |
| Heart, per cent of<br>Carcass | 0.658 ± 0.063<br>SE <sub>m</sub> 0.022<br>0.2 > P > 0.1 | 0.539 ± 0.099<br>SE <sub>m</sub> 0.035<br>0.5 > P > 0.2 | 0.435 ± 0.052<br>SE <sub>m</sub> 0.018<br>0.5 > P > 0.2 | 0.637 ± 0.056<br>SE <sub>m</sub> 0.019<br>P < 0.1 | 0.478 ± 0.055<br>SE <sub>m</sub> 0.020<br>0.5 > P > 0.2 |  |

SE<sub>m</sub> means standard error of the mean. Values of P less than 0.05 are considered statistically significant.

*Size of Muscle Fiber of the Hearts in Twenty-Four Hour Experiment*—It was not expected that the relatively small gross increase of mass of muscle substance of the heart at the end of a twenty-four hour experiment, when distributed over most or all of its muscle fibers, would manifest itself by an increase in the thickness of a muscle fiber at any one point as measured by an ocular micrometer. This was borne out when 100 muscle fibers of each of 2 control and 2 experimental hearts were measured. The average thickness of the muscle fibers was 41 ± 10 microns and 48 ± 11 microns, respectively, for the 2 control hearts and 41 ± 0.9 microns and 48 ± 1.1 microns, respectively, for the experimental hearts.

*Degree of Cardiac Hypertrophy Within Experimental Periods of from Two Days to Sixteen Days*—The results obtained in following the degree of hypertrophy of the heart under a salt regimen beyond the first day are listed in table 4. It will be noted that, except for the four day period, in all instances the experimental wet heart weight-body weight ratio was statistically higher than the control ratio. While the percentage increase in the experimental ratio was not steadily progressive in the manner of a growth curve, nevertheless, a fairly stable degree of maximal cardiac hypertrophy was reached under these experimental conditions within nine days.

Although in almost every instance the dry weight of the heart expressed as a percentage of its wet weight was less in the experiments, the differences were not statistically significant with two exceptions. In one of these exceptions, when fat-free values were obtained, the fat-free substance of the heart expressed as a percentage of its fat-free wet weight, although less than that of the control, was, none the less, of no statistical significance. This indicates that the increase in

TABLE 4—Heart Weights and Degrees of Hypertrophy During Experimental Periods of Two to Sixteen Days

|                | Days | Animals | Body Weight,<br>Gm | Wet Heart<br>Weight,<br>Gm | Wet Heart<br>Weight,<br>Body Weight,<br>per Cent                  | Dry Heart<br>Weight,<br>Wet Heart<br>Weight,<br>per Cent | Fat Free<br>Dry Heart Wt<br>Fat Free<br>Wet Heart Wt<br>per Cent * |
|----------------|------|---------|--------------------|----------------------------|---|--|--|
| Control        | 2    | 11      | 263.7 ± 28.5       | 1.588 ± 0.227              | 0.602 ± 0.051   | 26.219 ± 1.392   | 18.864 ± 0.626*  |
| Experimental   |      | 10      | 258.4 ± 36.7       | 1.936 ± 0.491              | 0.746 ± 0.138<br>P < .01<br>23.9% increase<br>over controls       | 25.083 ± 1.111<br>1 > P > .05                            | 18.388 ± 0.389*<br>2 > P > .1                                      |
| Control        | 3    | 12      | 252.3 ± 30.6       | 1.576 ± 0.179              | 0.626 ± 0.042   | 25.333 ± 1.953   | 19.742 ± 0.218*  |
| Experimental   |      | 12      | 257.3 ± 27.9       | 1.758 ± 0.198              | 0.684 ± 0.045<br>P < .01<br>9.2% increase<br>over controls        | 25.611 ± 3.559<br>P no<br>significance                   | 19.254 ± 2.415*<br>7 > P > .6                                      |
| Control        | 4    | 6       | 303.1 ± 12.5       | 2.043 ± 0.179              | 0.674 ± 0.047   | 26.499 ± 2.267   |  |
| Experimental   |      | 6       | 317.1 ± 17.6       | 2.305 ± 0.205              | 0.727 ± 0.057<br>2 > P > .1<br>7.9% increase<br>over controls     | 24.106 ± 1.740<br>1 > P > .05                            |  |
| Control        | 5    | 12      | 264.1 ± 30.8       | 1.599 ± 0.173              | 0.608 ± 0.051   | 25.869 ± 1.956   | 19.442 ± 0.783*  |
| Experimental   |      | 11      | 241.9 ± 11.5       | 1.969 ± 0.233              | 0.716 ± 0.035<br>P < .01<br>17.7% increase<br>over controls       | 22.765 ± 2.368<br>P < .01                                | 18.432 ± 1.221*<br>2 > P > .1                                      |
| Control        | 7    | 5       | 239.7 ± 15.8       | 1.499 ± 0.079              | 0.627 ± 0.040   | 23.833 ± 1.459   |  |
| Experimental   |      | 6       | 242.5 ± 18.9       | 1.697 ± 0.174              | 0.699 ± 0.049<br>0.5 > P > .02<br>11.7% increase<br>over controls | 22.333 ± 1.470<br>2 > P > .1                             |  |
| Control        | 9    | 4       | 329.0 ± 8.5        | 1.965 ± 0.086              | 0.597 ± 0.024   | 23.125 ± 1.263   |  |
| Experimental   |      | 5       | 320.2 ± 43.9       | 2.447 ± 0.471              | 0.762 ± 0.061<br>P < .01<br>27.5% increase<br>over controls       | 23.303 ± 2.562<br>P no<br>significance                   |  |
| Control        | 11   | 5       | 253.6 ± 12.0       | 1.493 ± 0.067              | 0.590 ± 0.040   | 23.185 ± 1.975   |  |
| Experimental   |      | 6       | 258.3 ± 11.5       | 1.869 ± 0.123              | 0.739 ± 0.059<br>P < .01<br>25.2% increase<br>over controls       | 20.516 ± 1.358<br>0.5 > P > .02                          |  |
| Control        | 12   | 5       | 299.7 ± 12.1       | 1.748 ± 0.102              | 0.584 ± 0.034   | 22.469 ± 1.187   | 19.018 ± 0.539   |
| Experimental   |      | 5       | 326.7 ± 46.7       | 2.326 ± 0.489              | 0.713 ± 0.109<br>0.5 > P > .02<br>22.2% increase<br>over controls | 21.499 ± 1.585<br>4 > P > .3                             | 18.450 ± 1.251<br>9 > P > .8                                       |
| Control †      | 15—  | 5       | 359.4 ± 40.0       | 2.045 ± 0.139              | 0.575 ± 0.074   | 20.065 ± 1.040   |  |
| Experimental † | 16   | 6       | 362.2 ± 26.7       | 2.904 ± 0.481              | 0.804 ± 0.142<br>P < .01<br>39.9% increase<br>over controls       | 19.975 ± 1.154<br>9 > P > .8                             |  |

\* The values were obtained on 6 birds

† The birds were cockerels

cardiac mass is a valid one, bearing with it not only an increase in total water but also an increase in the solid or fat-free solid substance

#### COMMENT

The data presented appear to prove in convincing fashion that, following a nine hour exposure to a high salt diet, the animals had hypertrophying hearts when examined at the end of twenty-four hours. This

is indicated by the statistically significant higher wet heart weight-body weight ratio, as well as by the dry the fat-free dry and the fat-free wet weight ratio of heart to carcass. There is an increase in the total nitrogen of the experimental hearts and in the ratio of total nitrogen of heart to total nitrogen of carcass. There seems to be little doubt that within the short period of twenty-four hours there had been an increase in the protoplasmic substance of the cardiac muscle fibers. Although there was in general a significant increase in the total amount of water of the heart, from our data this increase would appear to be predominantly intracellular. It has long been known that exercised striate muscle takes up water, and Loeb<sup>16</sup> assumed that it was due to increased osmotic pressure leading to absorption of water within the muscle fibers. The volume of muscle fiber was thereby increased and this was followed (or accompanied) by deposition of new material. This would appear to be the case in the present experiments. The interstitial edema of the hearts noted by Hitchings and associates,<sup>4</sup> which abruptly disappeared after the third day, may well have been due in great part to disturbances of coronary circulation resulting from the experimental aortic insufficiency.

Although the volume output of the heart of the experimental animals was not directly determined, there is reason to believe that the mechanism underlying hypertrophy in these experiments consists of dilatation of the cardiac chamber with probably an increased output per stroke. It has been our experience with hypertrophy of the heart in birds whose high salt intake was of longer standing that the chambers of the heart were considerably dilated. This was observed when the heart was exposed under anesthesia while still beating actively and also after removal, the chambers were larger than those of the controls, and their walls were hypertrophied. In the course of the present experiments attempts were made to detect such dilatation of the chambers of the heart by means of roentgenograms. It was found that it was impossible to outline the shadow of the chick's heart on a roentgenogram. Under the fluoroscopic screen, however, Dr. Roger A. Harvey, of the department of radiology, was able to determine roughly the transverse cardiac diameter when guided by the rapid flicker of the pulsations of the heart. It was found that the transverse cardiac diameter of a group of animals at 2 p.m. on the day before the experiment varied from 1.8 to 2.1 cm., whereas at 2 p.m. on the first day of the experiment the cardiac diameters of the same birds varied from 2.2 to 2.5 cm., an approximate increase of 19 per cent. Electrocardiographic tracings were obtained by Dr. Irwin R. Callen at the same time. There was little difference in the tracings on the day of the experiment as contrasted with those of the control period except for a change in voltage in a few instances in which either

16 Loeb, J. *Arch. f. d. ges. Physiol.* 56:270, 1894

the T wave or all complexes were lower on the day salt was administered. The cardiac rate in the preexperimental period was on the average 438 beats per minute, while in the afternoon of the experimental day it averaged 422 beats per minute. There was evidently little change in heart rate following the administration of salt. In view of the apparent cardiac dilatation observed fluoroscopically, it might be implied that with nearly complete emptying of the chambers there was an increase in cardiac output per beat.

There are two inferences that can be drawn from the observations that substantial cardiac hypertrophy can be demonstrated at the end of twenty-four hours after a brief intake of a high salt diet. The first of these is that cardiac hypertrophy cannot be a delayed process. Nor is it one that is in any way concerned with cardiac reserve. It represents rather an immediate response to Starling's law of increased output of energy of the heart, i.e., as soon as the cardiac muscle fibers are stretched or lengthened beyond their customary or physiologic range, the mechanism of hypertrophy is set into play. The second inference is that since brief periods of increased output of energy of the heart per stroke must be common in daily life and with it variations in the amount and the duration of such increases, cardiac mass is not stable but in a state of flux. There are probably periods of increase in cardiac mass, albeit small, as well as, undoubtedly, of regression toward the norm whenever the energy output of the heart returns to normal.

The present observations also emphasize the fact that a heavy load imposed on an otherwise sound heart with no disturbance of the coronary circulation will lead to a limited degree of hypertrophy, the maximal peak of which is reached in a matter of a few days. Aside from that due to the load (i.e., the degree of hypertension or of valvular stenosis or insufficiency), greater degrees of hypertrophy, with or without a slower rate of appearance and attainment of a maximum, are to some extent experimentally but particularly clinically in a great part dependent on myocardial damage or incompetence. This in the main is due to the direct effect of the disease process on the myocardium and/or the indirect effect of disturbances of coronary circulation. That there is however, individual variation in the ability of the myocardium to handle a given load is indicated by our repeated observations that degrees of hypertrophy of 50 to 100 per cent or more above the normal may be obtained in animals fed a high salt diet either during the time when they are rapidly growing or in older animals in the presence of apparent myocardial failure with persistent ascites.

#### SUMMARY AND CONCLUSIONS

Chicks standardized as to breed, age, sex and body weight were fed a 6 per cent sodium chloride food mixture over a period of nine

hours Observations were made on changes of cardiac mass at the end of twenty-four hours The progress of cardiac hypertrophy was studied also over a fifteen to sixteen day period

It is concluded from these observations that (1) a substantial degree of cardiac hypertrophy, 7 per cent or more above the normal weight, can be demonstrated repeatedly within a twenty-four hour period The inference to be drawn from this is that cardiac hypertrophy is neither a delayed response to increased work of the heart per stroke, nor one that is concerned with cardiac reserve, but is an immediate response to the increased output of energy It would follow from this that cardiac mass in the normal individual is not stable or fixed but fluctuates with shifts in the work of the heart per beat beyond that which is customary for the individual (2) For any particular load in the presence of a sound myocardium and in the absence of any condition interfering with the coronary circulation the maximum degree of hypertrophy is reached within a few days

## CARTILAGE IN THE KIDNEY

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FROM the few reports in the literature one might suppose that the finding of cartilage in the kidneys is of rare occurrence. No reports were found in the American literature and only a few in the German.

Schaffer<sup>1</sup> in 1897 reported his examination of an 8 month, dead fetus. Besides various malformations of other organs there was absence of the left kidney. The right kidney was cystic and twice the normal size. It contained abortive renal structures, striated and smooth muscle, islands of cartilage and epithelial elements which he considered malformations and not neoplasm. Busse<sup>2</sup> in 1904 found three small islands of hyaline cartilage with primary cartilage cells in a cystic kidney. Borrmann<sup>3</sup> in 1913 described a cystic kidney with underdeveloped glomeruli, fetal connective tissue, smooth muscle and cartilage. Ruckert,<sup>4</sup> Meyer,<sup>5</sup> Nathanson,<sup>6</sup> Berner<sup>7</sup> and Staemmler<sup>8</sup> have also reported the presence of cartilage in polycystic kidneys. The cartilage was present in the renal capsule, beneath the capsule or deeper in the cortex.

In 1930, while studying the microscopic sections of a hypoplastic kidney, we observed islands of cartilage. Since that time we have encountered cartilage in 14 kidneys, all of which were hypoplastic. In only a few hypoplastic kidneys was cartilage not present. These

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From the Children's Memorial Hospital and the Ortho S A Sprague Memorial Institute Laboratories

1 Schaffer Arch f Gynak **53** 15, 1897

2 Busse, O Virchows Arch f path Anat **175** 442, 1904

3 Borrmann, R, in Beitzke, H, and Bennecke, A Handbuch der allgemeinen pathologischen Anatomie des Kindesalters, Weisbaden, J F Bergmann, 1913

4 Ruckert, A Ueber Cystenieren und Nierencysten, in Festschr f Orth, Berlin, August Hirschwald, 1903, p 475

5 Meyer, E Virchows Arch f path Anat **173** 209, 1903

6 Nathanson Wien klin Wchnschr **16** 857, 1903

7 Berner Die Cysteniere Studien über ihre pathologische Anatomie, Jean, G Fischer, 1913

8 Staemmler Beitr z path Anat u z allg Path **68** 22, 1921



cartilage islands have never been found in the normal kidney. There has been no relation to infection. In some specimens large cysts were present, while in others there was cystic dilatation of the tubules. From moderate to severe disorganization of the renal elements, such as is usually found in the hypoplastic kidney, was present in all but one specimen. The greater the hypoplasia the greater the amount of connective tissue present. In only 1 instance have we found a miniature kidney which was approximately normal in its formation with normal structure other than the presence of islands of cartilage. The finding of cartilage has been so consistent in even routine sections that we believe it might well be found in nearly all hypoplastic kidneys with diligent search. The islands vary from small to large, are not seen grossly and from one to eight have been observed in one microscopic section. From this it may be assumed that many islands might be present in the whole specimen.

The ages of our patients varied from 4 days to 12 years. We have had no opportunity to examine kidneys of adults, but the same findings should prevail.

The 7 cases reported here are typical. Only the kidneys will be described.

#### REPORT OF CASES

**CASE 1**—The patient was a white boy aged 4 days. The right kidney and ureter were hypoplastic. The ureter was small and appeared not patent. The kidney was a nodular mass of reddish gray tissue 1 cm long and 0.5 cm in thickness.

The left kidney was moderately enlarged. The renal pelvis was enlarged, and the ureter was dilated and tortuous. The bladder likewise was enlarged and hypertrophied. The right ureter entered a small diverticulum and there was another diverticulum in front of the left ureteral orifice. In the posterior part of the urethra an iris diaphragm was present.

Microscopic examination of serial sections of the right ureter showed it to be small, but patent, with no evident opening into the bladder diverticulum. In the kidney there was evident hypoplasia with widespread fibrosis, marked dilatation and irregularity of the tubules, which were few and showed varying degrees of degeneration. Most of the glomeruli showed partial hyalinization, only a few appeared normal. In one section two areas of cartilage were present, one in the capsule and the other just beneath the capsule. The cells were small and fairly closely packed together, with less matrix than usual.

In the left kidney there was pyelonephritis, and in the left ureter and bladder there was acute suppurative infection.

A postmortem blood culture revealed hemolyzing *Staphylococcus aureus* in pure growth. Cultures of the urine and of pustular lesions of the skin made before death revealed hemolytic *Staph aureus* and hemolytic streptococci.

No other congenital anomalies were present.

**CASE 2**—A 6 day old white girl had a hypoplastic left kidney, measuring 1 by 0.5 cm. It was gray-red and appeared nodular because of the presence of many small cysts. The left ureter was made up of only a few fibrous strands.

The right kidney was about two-thirds normal size, weighing 10 Gm. it was irregular in outline, and there was a fairly widespread hemorrhagic suppurative necrosis. The renal pelvis and ureter showed only hyperemia of the mucosa.

The bladder was normal except for absence of the left ureteral orifice.

On microscopic examination of the right kidney there was widespread, marked erythrocytic and leukocytic infiltration, with well defined abscesses, especially at the upper and lower poles. No normal glomeruli were present, and the tubules showed varying degrees of degeneration and inflammatory changes.

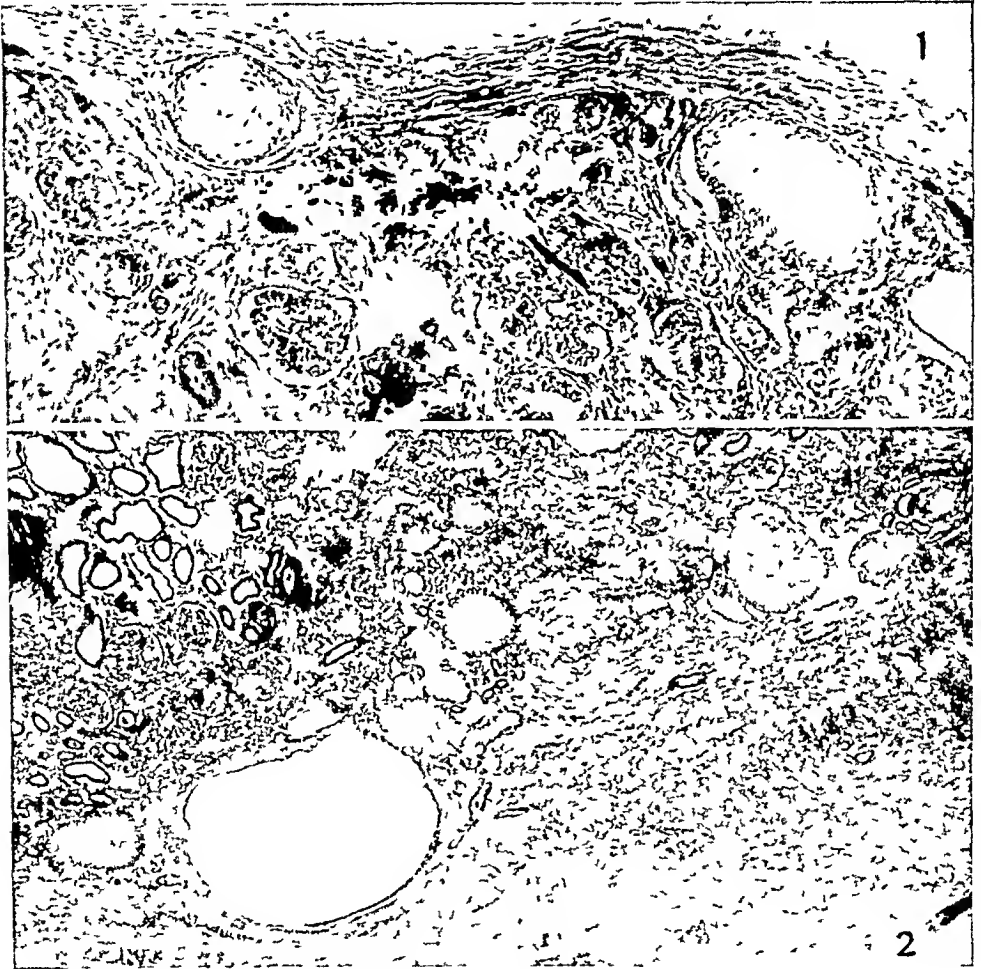


Fig 1 (case 1) —Two islands of cartilage, one in the capsule and one at the inner margin of the capsule ( $\times 85$ )

Fig 2 (case 4) —Five cartilage islands in one section. Note connective tissue, cystic dilatation of tubules and leukocytic infiltration ( $\times 35$ )

In the left kidney there was marked fibrosis of the parenchyma, with moderate fibrosis of the blood vessels, only a few widely scattered glomeruli were present, and they showed varying degrees of sclerosis and atrophy. The cysts were dilated tubules, which were numerous and irregular in outline. No normal tubules were present. There were a few focal areas of hemorrhage, and there was widespread round cell infiltration. The capsule was fibrotic and thickened. In one section

there was one small area of cartilage beneath the capsule. It was made up of closely packed small cartilage cells.

No other congenital anomalies were present.

CASE 3—In a white girl aged 5 months, the left kidney was larger than normal, weighing 40 Gm. The left ureter was normal.

The right kidney was a red-gray nodular mass containing two cysts. It weighed 5 Gm and measured 1 by 0.5 by 0.5 cm. The renal pelvis and ureter were fibrous, and the ureter did not reach the bladder. No renal artery or vein was present.

Microscopically the left kidney showed only intense hyperemia and moderate enlargement of the glomeruli.

The right kidney had a very thick capsule. There was more connective than renal tissue. All the tubules showed cystic dilatation and degenerative changes and were lined with low epithelial cells. Only a few glomeruli were present, all with varying degrees of sclerosis, none being normal. In two sections there was a small area of cartilage with a thick capsule and closely packed small cells.

No other congenital anomalies were present.

CASE 4—A white boy 11 weeks of age had a small right kidney containing many small cysts and measuring 3 by 2.5 by 1 cm. The capsule did not strip. On section the kidney was composed of homogeneous tissue and the cortex and the medulla could not be differentiated. The pelvis was markedly distended with urine. The ureter was large and tortuous.

The left kidney was small, like the right. On section there were several small cysts and the cortex and the medulla could be differentiated. The pelvis and ureter were moderately dilated.

The urinary bladder was contracted and thick walled. In the membranous portion of the urethra there was a valve formed by folds of the mucosa originating at the colliculus.

Microscopic examination was carried out on one kidney only. There was extensive, widespread fibrosis with large and small cystic dilatations of the tubules. Few glomeruli were seen, and those present were hypertrophied. There were focal regions of round cell infiltration. The blood vessels showed varying degrees of fibrosis. Multiple islands of cartilage were seen, with eight islands in one section alone. These islands were scattered widely throughout the renal tissue.

The only other anomalies present were a three-lobed left and a four-lobed right lung and an accessory spleen.

CASE 5—The patient was a white girl 10 years of age. The right kidney was normal, weighing 125 Gm.

The left kidney was small, measuring 4 by 2.5 by 1.5 cm and weighing 10 Gm. The surface was made up of rounded cysts. The ureter was normal. The renal artery and vein were small.

A microscopic examination of the left kidney was made. There was marked widespread fibrosis with cystic dilatation and degeneration of the tubules. The gross cysts appeared to be dilated tubules, with the epithelial lining being flattened but intact. The fibrous tissue was dense and almost hyaline in regions. Many small fetal tubules were present in the fibrous stroma. The glomeruli were large and few, some were almost completely hyalinized. One section showed, deep in the kidney structure, a small island of deeply staining cartilage.

There was also a large ductus arteriosus.

CASE 6—A white girl 12 years of age had a left kidney, normal in size and weight, which on cut section presented the classic picture of chronic pyelonephritis. The ureter was of normal size.

The right kidney was extremely small, weighing 3 Gm and measuring 3 by 2 by 0.5 cm. It seemed to be made up of small cysts. On cut section there was no likeness to renal tissue. The color was dark red-gray with lighter areas, which represented small cysts. The capsule could not be stripped. The ureter was hypoplastic. The right renal artery was not present.

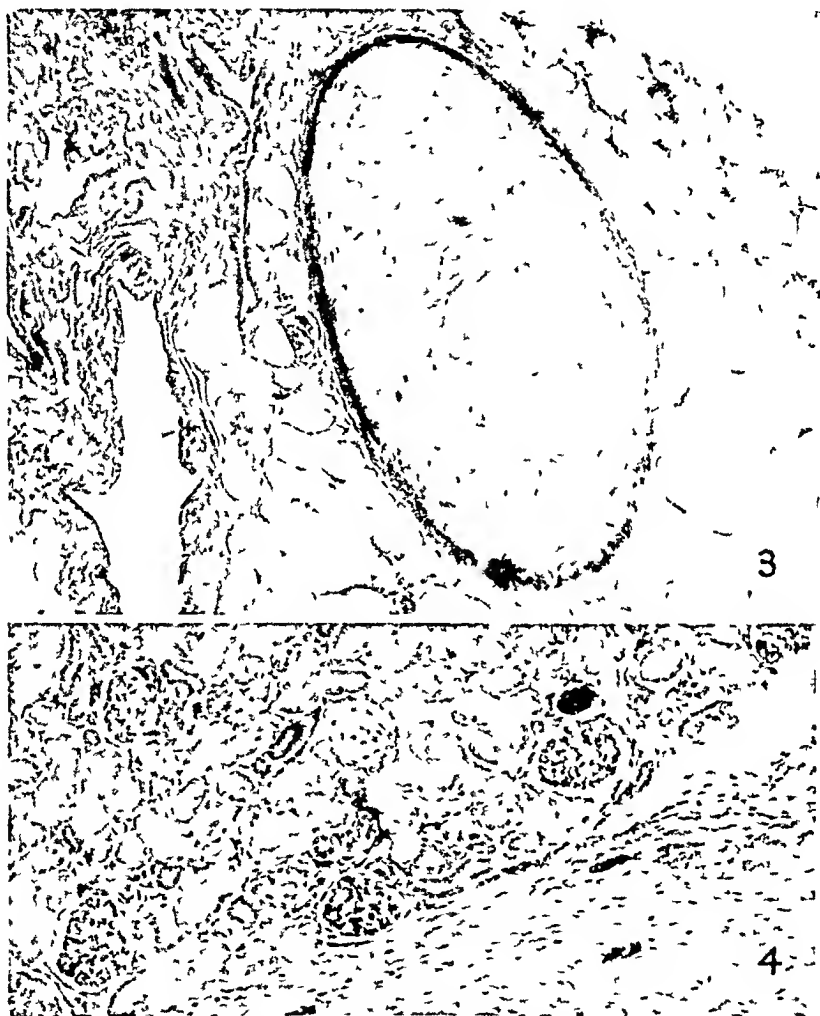


Fig 3 (case 6) —Large island of cartilage cells with a thick capsule in fatty tissue. The kidney was largely made up of connective tissue ( $\times 65$ ).

Fig 4 (case 7) —Small island of cartilage deep in the small right kidney. There was no other abnormality of the renal tissue ( $\times 90$ ).

Microscopic examination of the left kidney showed only the typical picture of advanced pyelonephritis.

The right kidney showed intense fibrosis throughout with some hyaline changes. The connective tissue (proliferation) was dense. Only a rare glomerulus that appeared to be functioning could be seen. Elsewhere the glomeruli were dense fibrous.

whorls, many of which were hyalinized. The tubules were prominent, many were dilated. Tubular degeneration was not impressive. There were varying degrees of sclerosis of the blood vessels. There were focal areas of leukocytic infiltration. A cartilage island was seen deep in the stroma near the pelvis in some fat. The cartilage cells were small and not closely packed together.

No other congenital anomalies were present.

CASE 7—The patient was a white boy aged 10 weeks. The left kidney was of normal size, weighing 29 Gm and measuring 5 by 3 by 3 cm. It appeared normal on cut section. The pelvis and ureter were normal.

The right kidney was hypoplastic, weighing 3 Gm and measuring 3 by 1.5 by 1.5 cm. On cut section the renal tissue appeared normal. The pelvis and ureter were normal.

Microscopically, there were no abnormalities of glomeruli, tubules, blood vessels or interstitial tissue of either kidney. Deep in the cortex of the right kidney one small island of cartilage was seen containing fairly large, closely packed cells.

No other congenital anomalies were present.

#### COMMENT

The islands of cartilage found in the kidney were small and were located in the capsule, just beneath the capsule, deep in the cortex and, in 1 instance, in fat near the hilus. The presence or the significance of these islands is not easy to explain. Schaffer<sup>1</sup> described the cartilage islands as having the characteristics that have been postulated for the primary anlage of cartilage. The cartilage cells are close to one another and have a large amount of protoplasm, and there is absence of ground substance. Berner<sup>7</sup> and Staemmler<sup>8</sup> favored the hypothesis that they may be cell rests or a displacement of derivatives of the primary vertebral myotomes and lateral plates, small foci of which become broken off. A more logical explanation would seem to be that they are due to metaplasia occurring in the mesodermal embryonal tissue that goes to the formation of the kidney.

As far as we have been able to gather from the literature, cartilage islands have not been found in the normal kidney. The cases reported in the German literature (as cited in the foregoing pages) have occurred in polycystic kidneys and in adenosarcoma and its metastasis. In our own experience these cartilage islands have always been in hypoplastic kidneys with or without cysts. Except in 1 instance there has been from moderate to complete disorganization of all of the renal elements. We have never found cartilage in a renal tumor. This may be due to an oversight, as the islands are small and may not have been present in the sections studied, but it seems that a chance finding would have occurred in the many tumors examined. Neither have we seen a neoplasm or neoplastic cells in any of the hypoplastic kidneys studied.

As islands of cartilage have in nearly all cases been found in kidneys with congenital malformations it seems likely that in the disorganization of the renal tissue the cartilage might well be a metaplastic

response of embryonal mesoderm which has failed to differentiate itself into its proper renal elements from the beginning. This might explain why so much connective tissue is found in hypoplastic kidneys, as well as smooth and striated muscle. The kidney is of mesodermal origin and there is a great potential, therefore, for the development of connective tissue, of which cartilage is one form. Gruber<sup>9</sup> expressed the belief that this early differentiation begins to take place before the fourth week of life in the embryo. He also expressed the theory that cartilage and muscle tissue in the kidney may be regarded as prosoplastic formations. Under the influence of a growing irritation (disorganization) a prosoplastic hamartoma and even a hamartoblastoma might develop. He further stated that such a hypothesis could be appropriated for the mixed tumors of the kidney.

#### SUMMARY

In congenital hypoplastic and in congenital cystic kidneys islands of cartilage are apparently not uncommon. Their presence is most likely due to the disorganization of the embryonal renal tissue which occurs with kidney malformations.

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<sup>9</sup> Gruber, B., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol. 6, p. 35.

# LATE EFFECTS OF RADIUM AND PLUTONIUM ON BONE

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AS IS WELL known, irradiation produces devitalized bone and at times an overgrowth of bone together with fibrous and gelatinous marrow (Regaud<sup>1</sup>, Ewing<sup>2</sup>, Phemister<sup>3</sup>, Gall, Lingley and Hilcken<sup>4</sup>). It has also been demonstrated that the introduction of bone-seeking isotopes results in marked proliferative and degenerative changes in bone and marrow (Martland and Humphries<sup>5</sup>, Martland<sup>6</sup>, Thomas and Bruner<sup>7</sup>, Dunlap, Aub, Evans and Harris<sup>8</sup>). Osteogenic sarcomas have been found after external irradiation and have been studied in some detail after internal irradiation (Martland<sup>6</sup>, Sabin, Doan and Forkner<sup>9</sup>, Dunlap, Aub, Evans and Harris<sup>8</sup>, Evans, Harris and Bunker<sup>10</sup> and others). The details of the changes in bone after irradiation vary somewhat with the type of agent, dosage and especially with the animal species (see review by Gates<sup>11</sup>).

Acute and subacute sequelae of the introduction of relatively large amounts of a number of bone-seeking isotopes have been described by

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From the Argonne National Laboratory and the Department of Anatomy and Institute of Radiobiology and Biophysics, University of Chicago.

1 Regaud, C. *Compt. rend. Soc. de biol.* **87** 629, 1922.

2 Ewing, J. *Acta radiol.* **6** 399, 1926.

3 Phemister, D. B. *Am. J. Roentgenol.* **16** 340, 1926.

4 Gall, E. A., Lingley, J. R., and Hilcken, J. A. *Am. J. Path.* **16** 605, 1940.

5 Martland, H. S., and Humphries, R. E. *Arch. Path.* **7** 406, 1929.

6 Martland, H. S. *Am. J. Cancer* **15** 2435, 1931.

7 Thomas, H. E., and Bruner, F. H. *Am. J. Roentgenol.* **29** 641, 1933.

8 Dunlap, C. E., Aub, J. C., Evans, R. D., and Harris, R. S. *Am. J. Path.* **20** 1, 1944.

9 Sabin, F. R., Doan, C. A., and Forkner, C. E. *J. Exper. Med.* **56** 267, 1932.

10 Evans, R. D., Harris, R. S., and Bunker, J. W. M. *Am. J. Roentgenol.* **52** 353, 1944.

11 Gates, O. *Arch. Path.* **35** 323, 1943.

Heller<sup>12</sup> The long term chronic effects of small doses of plutonium (a pure  $\alpha$  emitter) and radium, with special reference to tumor production, have been reported by Brues, Lisco and Finkel<sup>13</sup> A sampling of the animals given injections for this chronic program was set aside for serial autopsy and it is on this material that the present paper is based We shall describe the bone changes observed in these serially autopsied mice during a period of from two months to a year after injection of small amounts of plutonium or radium

Since many of the previous studies in the literature were made on growing animals, it has not always been possible to separate the effects of radiation on compact and cancellous bone from those on the bone growth mechanism, including the cartilage at the metaphysis The effects of radium and plutonium on our mice—which were essentially adult animals—are manifested mainly in new formation of typical and atypical bone and in devitalization of old as well as new bone The absorption of bone previously reported in other species after introduction of certain isotopes either did not occur in our mice or was an inconspicuous part of the picture

#### MATERIAL AND METHODS

The effects of radium and plutonium administered in a single small dose two months to a year previously were studied in the femurs of about 100 male and female mice of the CFl strain as compared with those of untreated controls of the same age kept under identical laboratory conditions Radium was injected intraperitoneally, 0.3 microcurie per gram of body weight to one group, 0.03 microcurie per gram to another Plutonium was given intravenously, 0.03 or 0.003 microcurie per gram Autopsies, usually on groups of 4 or more treated mice and on several untreated controls, were performed at intervals starting two months after treatment with the higher dose and three months after, with the lower dose At the higher dose levels the survival time was shorter, and the latest interval at which autopsies were made was seven months for plutonium and five months for radium With the lower doses some animals were killed as long as eleven or twelve months later Tissues were fixed in formaldehyde-Zenker solution, embedded in pyroxylin (nitrocellulose), decalcified, cut and stained with hematoxylin-eosin-azure II for histologic study The description of the bone changes reported here is based primarily on sections of the femurs Vertebrae of these same animals were prepared similarly, and are described briefly for comparison of the effects on these and on the long bones The changes in the other organs will be described in another report

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12 Heller, M Histopathology of Irradiation from External and Internal Sources V Bone, National Nuclear Energy Series (Manhattan Project Technical Section), New York, McGraw-Hill Book Company, Inc, 1948, vol 22-I

13 Brues, A M, Lisco, H, and Finkel, M P Late Effects of Injected Plutonium on Mice and Rats, National Nuclear Energy Series (Manhattan Project Technical Section), to be published



## RADIUM

*Femurs of Mice Given 0.3 Microcurie per Gram*—Of the animals which received this dose, a number survived for five months, and autopsies were made at monthly intervals from two to five months. At two months the femur shows striking changes from the normal. In the epiphysis the bone is dead and the marrow gelatinous. The metaphysis is characterized by a great overgrowth of bone, so that the thin trabeculae of the normal spongiosa are now fused into an intricate network of irregularly arranged trabeculae which extend far into the adjacent marrow of the shaft. This bone is atypical in arrangement and staining. It is often coarsely fibrillated and in places deeply basophilic, in others acidophilic (fig 3). Most of the lacunas are empty, and there are practically no living osteocytes. In some areas the new bone is dense and fairly regular. But here, too, most of the lacunas are empty. In one specimen only, there are still prominent remains of the original trabeculae of the spongiosa, with calcified cartilage, but these are entirely surrounded by new, blue-staining and atypical fibrous bone. The diminished marrow spaces are gelatinous. The cortical bone of the shaft contains a fair number of empty lacunas (fig 1) and dead osteocytes. The new bone in the medullary cavity shows varying degrees of irregularity of arrangement and often is devoid of living osteocytes (fig 2). The marrow of the central portion of the shaft is hemopoietic.

The degree of damage in the epiphysis is the same at three months as at two. The metaphysis in some specimens is even more solidly filled with devitalized bone than at the earlier interval (fig 4). The new bone in the distal portion of the shaft extends for some distance into the medulla as an extension of the spongiosa and in part as an ingrowth from the endosteum of the diaphysis. Here a layer of osteoblasts is sometimes prominent along the surface of the bone. In addition to the encroachment of bone from the metaphysis into the marrow cavity of the shaft, there is occasionally medullary bone in the shaft at a considerable distance from the metaphysis and separated from it by hemopoietic marrow. This newer bone, in contrast to the older atypical bone of the metaphysis, contains many normal osteocytes, as well as some dead ones and empty lacunas, but differs from normal bone in the irregular arrangement of the bone cells. An osteoblastic layer is usually prominent. Some of this bone has been laid down recently, whereas some is quite dense. Except where it has been replaced by bone, most of the marrow of the shaft is hemopoietic at this time, except for one specimen in which gelatinous marrow extends deep into the shaft. In the middle of the shaft of this femur there is a small area of very atypical bone, fibrous but of varying density, closely associated with numerous spindle cells and in a few areas containing empty lacunas. Although this section was taken several months before the time when gross sarcoma is observed in similarly treated animals, this discrete bit of atypical bone is consistent with what one might expect of an exceedingly early sarcoma.

At four months two of the three specimens reveal extensive damage, with much dead and atypical bone in the metaphysis, although the relative amounts of devitalized bone and gelatinous marrow differ in the two. The bone of the shaft varies from the relatively smooth contours of normal bone to greater or lesser irregularities in the endosteal surface, with projections into the marrow cavity, in one instance forming a complete bridge across it. In the latter case the bone is compact but less regular in cellular and lamellar arrangement than normal bone, and in places exceedingly fibrous. Interspersed with the living osteocytes in this bone are some empty lacunas. An osteoblastic layer extends along part of the surface of this bone, the rest being covered by spindle cells.

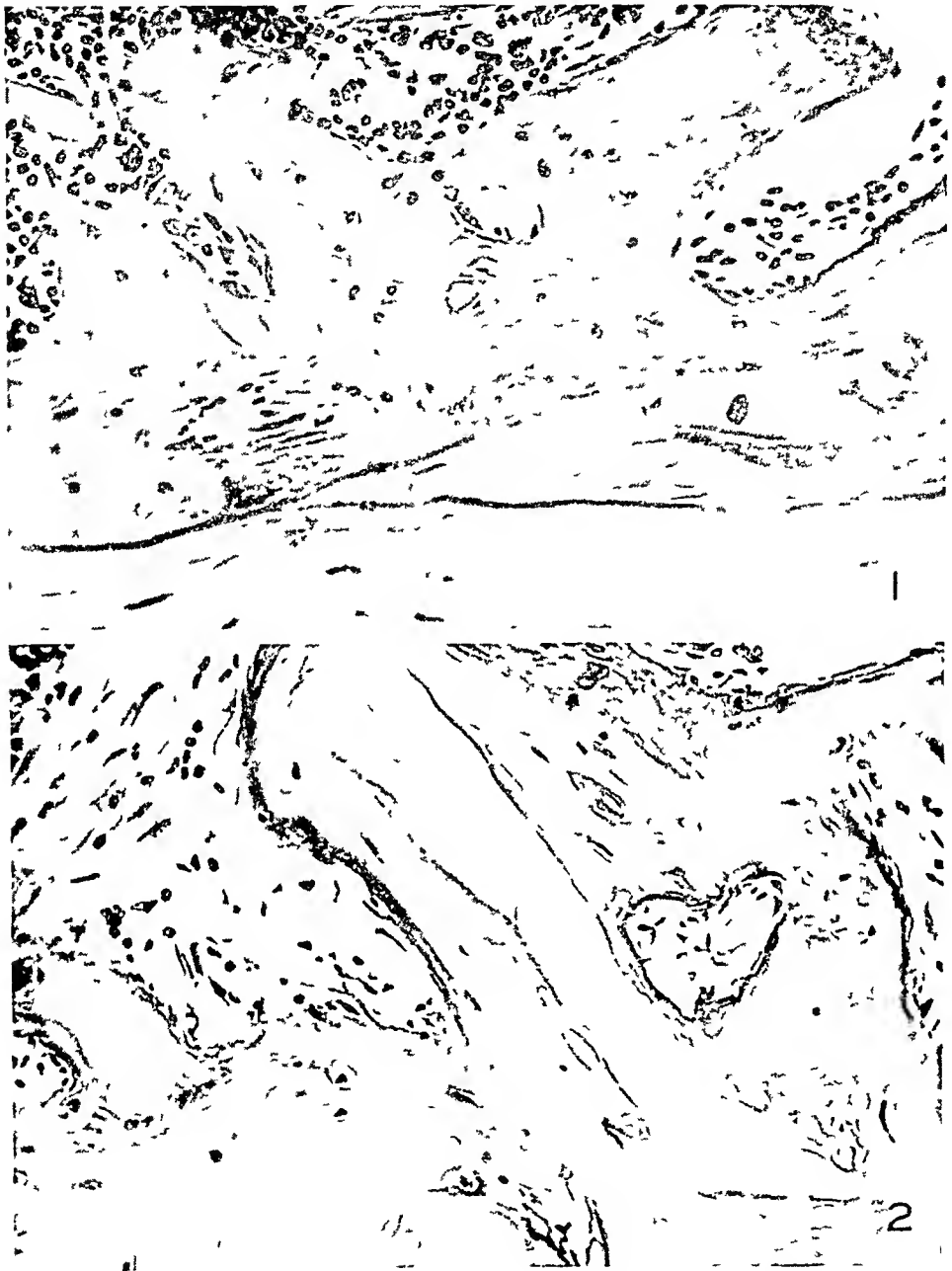


Fig 1—Shaft of mouse femur two months after intraperitoneal injection of 0.3 microcurie of radium per gram of body weight. It shows cortical bone, partially devitalized, with new bone growing from the endosteal surface into the marrow of the shaft.  $\times 283$

Fig 2—Shaft near metaphysis of the same femur as in figure 1. An advancing edge of bone is encroaching on gelatinous marrow. Note the empty lacunas and the irregular lamellation.  $\times 283$

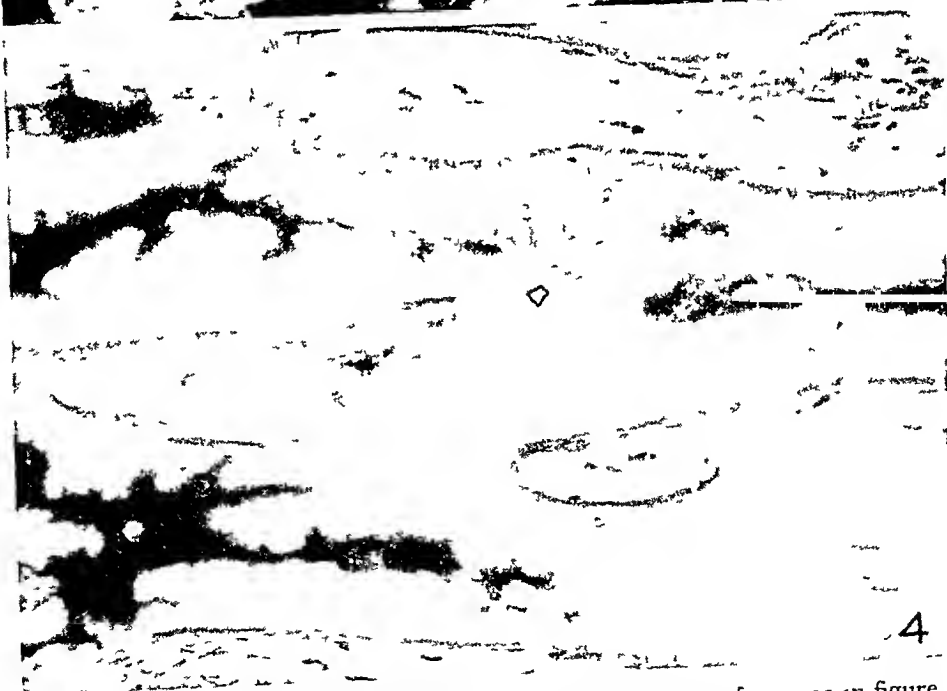
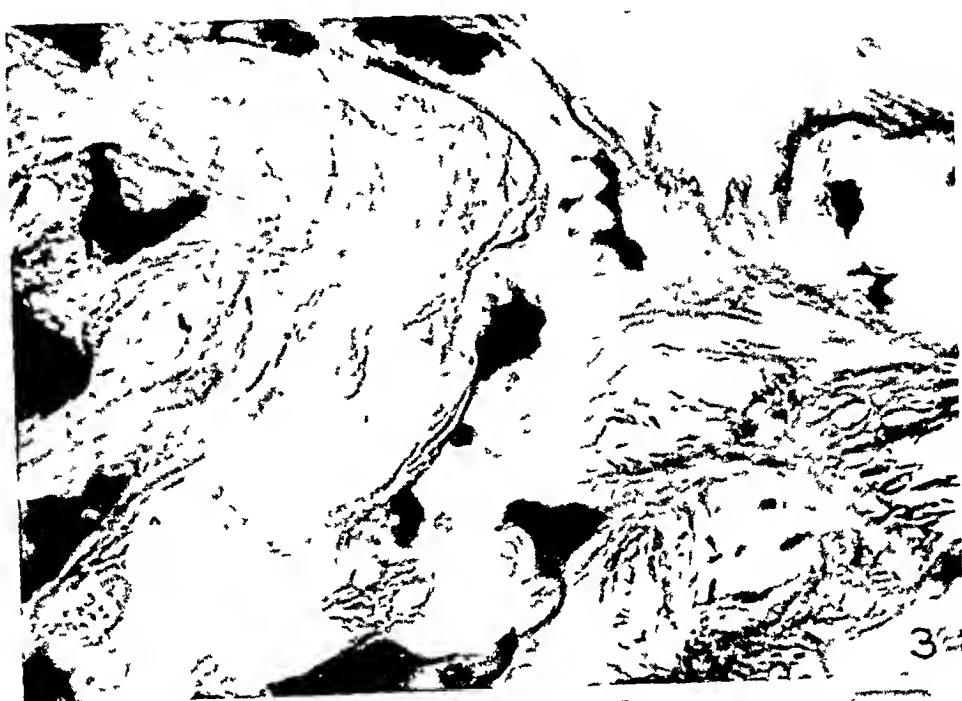


Fig 3—Metaphysis near the cartilage plate from the same femur as in figure 1. Note dark-staining, fibrous new bone between preexisting trabeculae. Calcified cartilage appears black.  $\times 295$

Fig 4—Metaphysis of mouse femur three months after injection of 0.3 microcurie of radium per gram of body weight. There are deep gray areas of new bone along the margins and in the substance of the trabeculae. The marrow is gelatinous. Cartilage remnants appear black.  $\times 295$

In the bone of the shaft empty lacunas occur fairly frequently, but are not confined consistently to either the endosteal or the periosteal portions of the bone. The marrow in the shaft is hemopoietic, even in the femur in which bone has grown across the marrow cavity, the area between this bone and that in the proximal end is filled with hemopoietic cells.

One of the three specimens at this interval shows much milder radiation effects than the others. Along the metaphysial surface of the cartilage plate extends a thick band of bone, most of it dead, with only a few scattered osteocytes near the shaft side. The marrow is hemopoietic.

The picture is much the same at five months as at four, except that in the metaphysis and the epiphysis the atypical bone is, in some specimens, less fibrous and more like compact bone, although with practically all lacunas empty. Again, the spaces between the trabeculae are filled with gelatinous marrow. The marrow of the shaft is hemopoietic. In one specimen a network of trabeculae of fairly normal-appearing bone arises from the endosteum, extending across the marrow cavity in several places.

*Femurs of Mice Given 0.03 Microcurie per Gram*—Mice which received this lower dose of radium were submitted to autopsy after three, five, seven and twelve and one-half months.

At three months the picture either is that of a normal older bone or shows some thickening of the trabeculae beneath the cartilage plate. In one femur the metaphysial bone is somewhat thickened, with the trabeculae tending to run parallel to the cartilage plate. In contrast to the mild changes in the other specimens at this interval, one femur resembles those at the higher dose level. Dead and atypical bone fill the metaphysis and extend well into the shaft, with dead osteocytes and empty lacunas prominent in the thickened and irregular shaft bone near the metaphysis.

Profound changes have occurred by five months. In some femurs the epiphysis is almost completely filled with dense bone. In the metaphysis, too, there is a great overgrowth of bone, some of its cells dead, in contrast to the living osteocytes of the epiphysis. In one specimen, beneath this bone inside the metaphysis, there is a peculiar dense fibrous tissue, denser than fibrogelatinous marrow but distinctly different from the early atypical fibrous bone formed after higher doses of radium. Unfortunately, we cannot tell whether this was calcified, since the block had been decalcified and no von Kossa preparations could be made. This tissue merges gradually with dense gelatinous marrow which extends into the portion of the shaft containing an anemic infarct. Proximal to this there is hemopoietic and some fatty marrow. Much of the cortical bone of the shaft is also dead.

Seven months after treatment the bone of the metaphysis is excessive in amount, that near the shaft is all dead, as is most of that in the rest of the metaphysis. However, in one specimen there is a thin layer of normal bone beneath the cartilage plate. Most of the diaphysial bone is dead, and it is usually thickened and, in places, irregular. In one specimen an anemic infarct occupies most of the metaphysis and shaft. Near the proximal end of the shaft the marrow is gelatinous, becoming fibrogelatinous in places. In the other specimen the marrow in the shaft is hemopoietic.

At twelve and one-half months the femurs of the 2 mice show little radiation damage. In one there has been closure of the epiphysial-metaphysial junction, there is no gelatinous marrow and only a little new bone. In the other there is only a slight overgrowth of bone in the epiphysis. We have no explanation for these apparently exceptional animals.

*Vertebrae*—Two months after the mice have been given the injection of 0.3 microcurie of radium per gram, the preexisting bone of the vertebrae is practically all dead and acellular. Blue-staining atypical fibrous bone like that formed in the femurs has developed around the trabeculae of the spongiosa. It varies in extent but is plentiful in all specimens. Most of this newer bone is also devitalized, though in portions of one specimen living osteocytes remain, irregularly arranged. In another there is little fibrous bone, but an atypical, dense, apparently homogeneous ground substance has developed in the marrow. The marrow itself is gelatinous and depleted, with only a sprinkling of hemopoietic cells.

At later intervals, too, most of the old bone and of the newer atypical fibrous bone is dead. The peculiar ground substance noted at two months is present in some specimens at three, four and five months. It is sometimes vacuolated, stains pinkish gray and bears no particular resemblance to bone or connective tissue. It occurs frequently in the neighborhood of hemopoietic cells, although the rest of the marrow may be gelatinous. The marrow within these vertebrae as a whole is gelatinous. Hemopoiesis, entirely absent from some specimens, varies in others from occasional small foci to large areas of activity.

A small giant cell granuloma is present in the connective tissue near a vertebra of a three month animal.

In a vertebra of a five month mouse there is an area of intense and somewhat atypical proliferation of osteoblasts.

After the lower dose of radium, 0.03 microcurie per gram, the vertebrae show only mild radiation effects three and five months after treatment. The bone is more compact than after the higher dose and more like the bone of femurs after injection of plutonium. Some of the lamellas are quite irregular, others show the normal, roughly parallel configuration. There are empty lacunas in the bone near the cartilage plate, but the cortex and some of the trabeculae contain mostly living osteocytes. The marrow is hemopoietic, with little depletion at three months and none at five. By seven months there are areas of dead bone with many empty lacunas. Overgrowth of bone has occurred and is quite extensive in some vertebrae. The marrow is depleted and gelatinous in places, but on the whole it is hemopoietic.

Three specimens are leukemic. In 2 of these the leukemia is extremely early, in the third it is somewhat more advanced. Like the femurs, the vertebrae of the 2 mice examined at twelve and one-half months resemble the controls, except that one has definite leukemia, the other possibly very early leukemia. One of the seven month untreated controls is also leukemic.

#### PLUTONIUM

*Femurs of Mice Given 0.03 Microcurie per Gram*—In the series in which 0.03 microcurie of plutonium per gram was injected intravenously, the first autopsies were made two months after treatment. At this time there is some overgrowth of bone in the epiphysis. The trabeculae are thickened, in places forming irregular primitive haversian systems. There is a great overgrowth of bone in the metaphysis and extending well into the shaft, where it frequently forms a thick meshwork across the marrow cavity (fig 5). Osteoblasts are numerous, some of them enlarged. A few osteoclasts are also present. Although some trabeculae are devitalized they are covered with a layer of osteoblasts or spindle-shaped cells. The metaphyseal bone varies in degree of abnormality from specimen to specimen.

Varying numbers of the osteocytes are dead. But in no specimen do empty lacunas predominate as in the bones of the animals receiving 0.3 microcurie of radium per gram.

Changes have also occurred in the bone marrow. In the epiphysis the marrow is mainly gelatinous or fatty, with only scattered hemopoietic cells or one or two foci of them. In the metaphysis the marrow is almost entirely gelatinous, with many spindle cells and usually only scattered hemopoietic cells, mostly small granulocytes. Only in one specimen is there hemopoiesis close to the cartilage

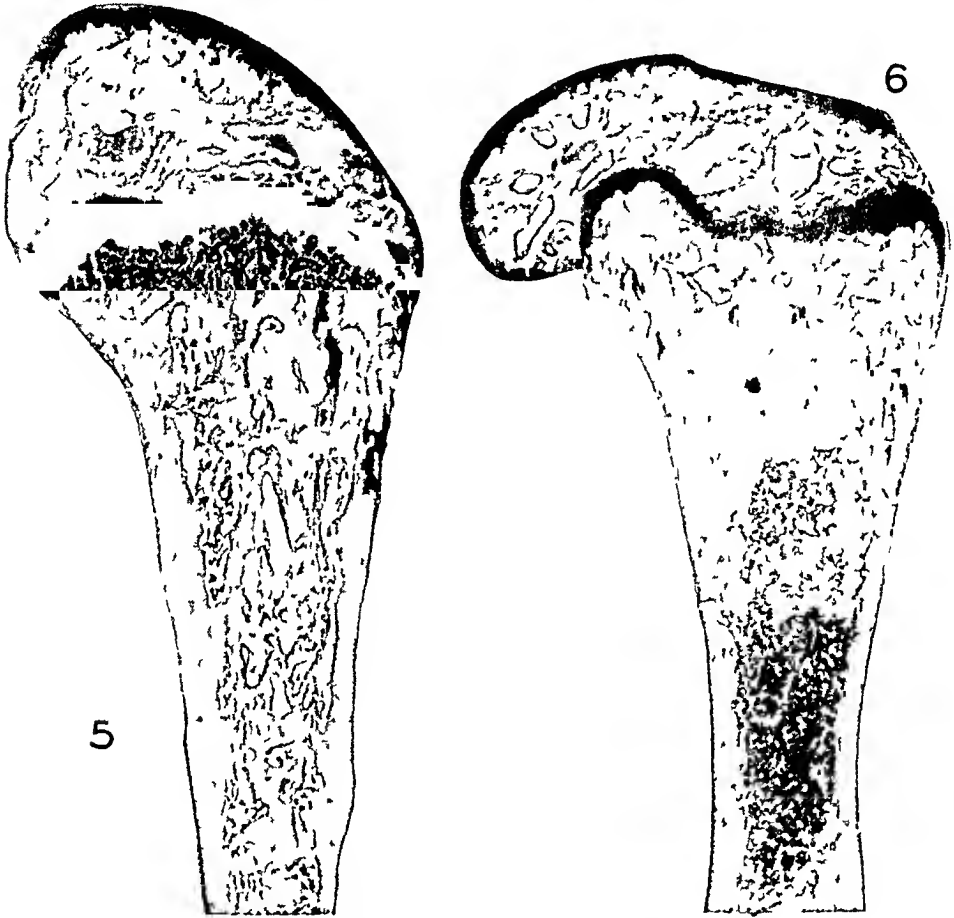


Fig 5—Femur of a mouse two months after intravenous injection of 0.03 microcurie of plutonium per gram of body weight. The overgrowth of bone practically fills the cavity of the shaft.  $\times 15$

Fig 6—Femur of a mouse seven months after injection of 0.03 microcurie of plutonium per gram of body weight, showing extensive osteosclerosis and two areas of hemopoietic marrow.  $\times 15$

plate. In the interstices between the trabeculae inside the shaft, the marrow varies from gelatinous and fatty to hemopoietic. Only the midportion of the shaft contains hemopoietic marrow.

At three months there is osteosclerosis in the epiphysis, with trabeculae of bone dead in some specimens and not in others.

The extent of overgrowth in the metaphysis is about the same as at the earlier interval. However, in one specimen at this time the bone contains mostly



Fig 7—Femur of a mouse three months after injection of 0.03 microcurie of plutonium per gram of body weight. Atypical, basophilic, fibrous bone in metaphysis, interspersed with gelatinous marrow. Cartilage appears black.  $\times 324$

Fig 8—Femur of a mouse five months after injection of 0.03 microcurie of plutonium per gram of body weight, showing new bone arising from endosteum in the central portion of the shaft. Note covering layer of basophilic osteoblasts.  $\times 81$

empty lacunas, and surrounding many of these spicules is an atypical, blue-staining bone, practically free of lacunas. Close to the cartilage plate, especially this bone is particularly atypical, fibrillar and deeply stained (fig 7).

The marrow in the epiphysis as well as in the metaphysis is almost wholly gelatinous. Toward the shaft there is sometimes more hemopoiesis. The shaft itself usually contains hemopoietic marrow, in the specimen showing the greatest damage in the metaphysis, the marrow in the shaft contains giant hemocytoblasts and a number of degenerating granulocytes.

By four months there have been further changes. The metaphysial bone, much of it devitalized, has increased in amount and is continuous with the cortical bone of the shaft. Atypical fibrous bone surrounds many of the trabeculae of compact or spongy bone, the latter varying greatly from specimen to specimen, and even from one area of the metaphysis to another in the same section. The bone extends farther into the shaft than at three months. Osteoblasts are absent from the metaphysis near the cartilage plate but are present nearer to the shaft, where they surround some bands of devitalized as well as atypical bone. In one specimen there is some new bone covered with osteoblasts near a hemopoietic area just beneath the cartilage plate. The epiphysial bone is thickened and irregular, and contains some empty lacunas and dead osteocytes.

The epiphysial marrow at this time is fatty, with a layer of gelatinous marrow bordering the spicules of bone and containing scattered small groups of late hemopoietic cells, mainly granulocytes. The small marrow spaces in the metaphysis are gelatinous. In a few of these, in addition to spindle cells, there are heterophilic leukocytes, in others, eosinophilic leukocytes. The marrow of the shaft contains widened sinuses in some portions and erythropoietic and myelocytopoietic cells in others. The hemocytoblasts have large, opaque nucleoli, and an occasional dead cell is present. No mitoses are seen in some specimens, in others, in which mitoses are numerous, the cells are mainly late forms.

Five months after treatment the femurs of the different animals vary considerably, suggesting a spotty distribution of the plutonium. All show a marked overgrowth of bone in the metaphysis. This is an irregular compact bone, devitalized in places and usually bordered by atypical fibrous bone. In some specimens this bone forms a wide band beneath the cartilage plate, in others it spreads well into the shaft. The inner surface of the shaft bone may be relatively smooth, or it may send projections of spongy and compact bone into the marrow cavity. An early stage of this process is seen in figure 8. The spicules of bone inside the cavity of the shaft are bordered by a layer of osteoblasts. There is some increase in bone in the epiphysis, often with dead osteocytes and empty lacunas, and surrounded or replaced by atypical fibrous bone.

The marrow in the epiphysis is gelatinous or fatty, with few or no hemopoietic cells. Most of the smaller marrow spaces in the metaphysis are filled with gelatinous marrow and spindle cells, whereas the larger ones contain many hemopoietic cells. Inside the shaft the spicules of bone are surrounded by gelatinous marrow. The marrow of the rest of the shaft is hemopoietic. There is some erythropoiesis, but most of the cells are myelocytes.

By seven months the process is still further advanced. The bone is exceedingly dense in both the epiphysis and the metaphysis (fig 6). The spongiosa consists of old bone and many successive layers of new bone, all dead in some animals and nearly so in others.



In the diaphysis bone from both the metaphysis and the endosteum has invaded most of the marrow cavity, some of it resembles normal dense cortical bone, but for the most part it is atypical in arrangement and varies from spongy bone to broad trabeculae of irregular compact bone with only small marrow spaces (figs 9, 10 and 11) A few of these spicules are covered by osteoblasts, more of them, by spindle cells Scattered osteoclasts are also present The cortical bone of the shaft proper is quite thick and irregular on its endosteal surface, usually with a lining of osteoblasts

In one specimen the atypical bone in the marrow of the shaft is very cellular, composed of somewhat atypical spindle and round cells with only a little intercellular substance Since this specimen represents a dose and time at which sarcoma is frequently observed, this atypical bone may well be an early sarcoma (fig 12) In the marrow of the shaft of this same specimen there is a second area of atypical bone, characterized by an excessive number of osteoblasts and spindle cells, some atypical, and one giant osteoblast with an enormous attraction sphere

The extensive bone growth which has taken place by seven months leaves only insignificant marrow spaces These are gelatinous in the metaphysis, gelatinous and fatty in the epiphysis In the shaft many of the marrow spaces are small and gelatinous, the few remaining larger ones contain groups of hemopoietic cells between the sinuses There is some debris, as well as many degenerating cells Hemocytoblasts and large myelocytes contain clumped chromatin

*Femurs of Mice Given 0.003 Microcurie per Gram*—Another group of about 30 mice was given by injection 0.003 microcurie of plutonium per gram, or one-tenth the dose administered to the series described in the foregoing section Three months after treatment the overgrowth of bone in the metaphysis is much less than with the higher dose at either two or three months Osteoblasts are prominent except where spindle cells replace them Empty lacunas and dead osteocytes are rare except in some spicules of one specimen The marrow of the metaphysis as well as that of the shaft is hemopoietic, although a number of the cells, especially the hemocytoblasts, show signs of degeneration

In the several five month specimens the damage varies widely in degree and in location In one there is overgrowth of bone beneath the cartilage plate, in another this region is hemopoietic, while lower in the metaphysis bone has grown in from the cortical bone This devitalized bone within the marrow cavity has some osteoblasts along the surface and is surrounded by gelatinous marrow In this femur the marrow is hemopoietic in the central portion of the shaft and in that part adjacent to the metaphysis, between these parts there is a large area of gelatinous marrow There is also an infarct in the shaft, with fibrosis around its margins In another femur new bone from the shaft has invaded the marrow cavity in a number of places, sometimes forming an almost complete bridge across it

In one specimen the outer layers of the shaft have the normally pink-staining matrix, while the inner layers stain blue Extending from the cortical bone into the marrow cavity is a large, irregular trabecula with scattered bone cells and haversian canals The matrix is irregular in staining in places there are long pink, blue and gray stained areas, some irregular in outline, some blue throughout In one area the lamellas are very irregularly arranged, roughly centering about thin-walled blood vessels and little marrow

At seven months there is a great overgrowth of bone in both epiphysis and metaphysis This bone is also irregular in arrangement Unevenness in the

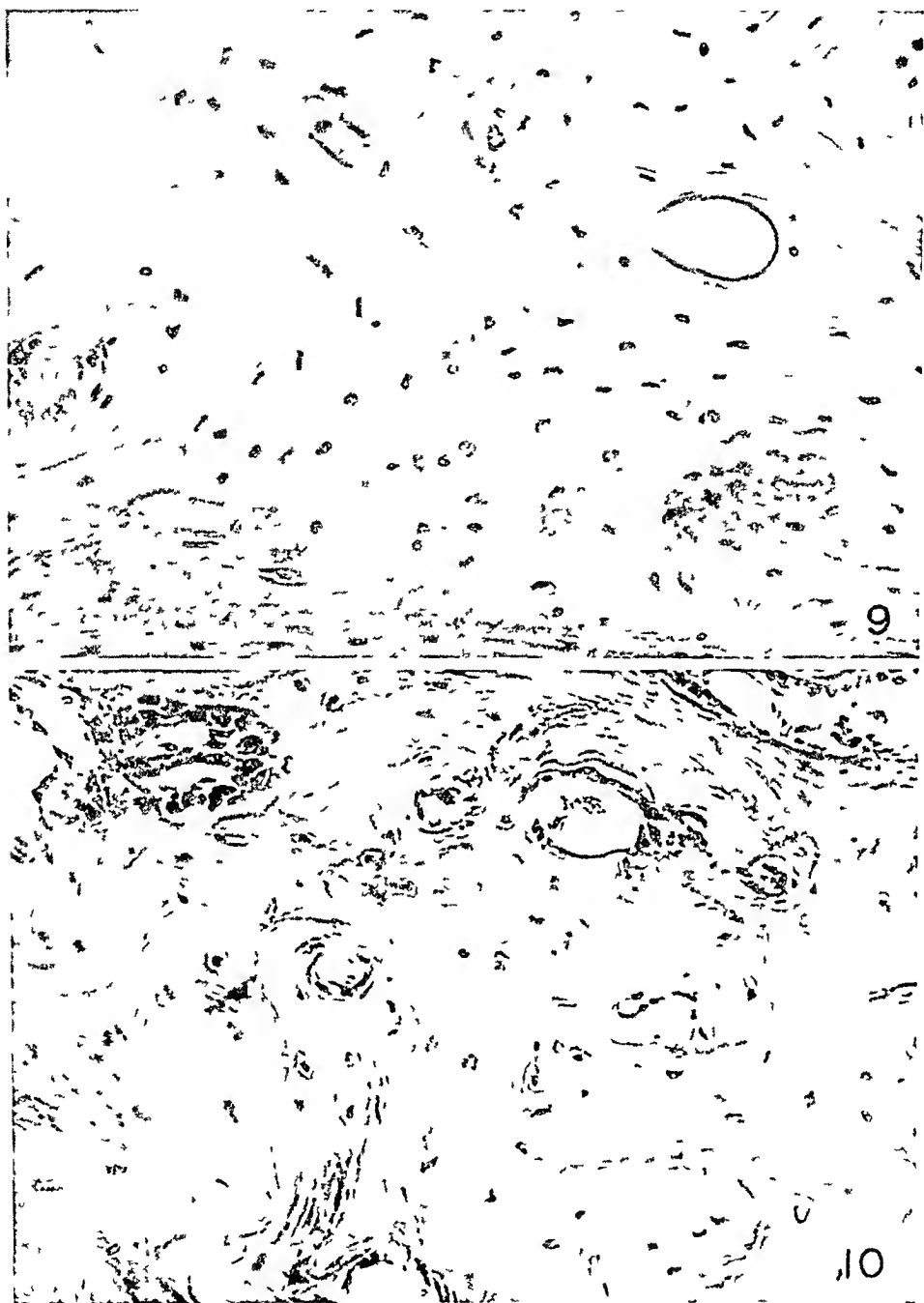


Fig 9—Area of shaft from the section shown in figure 6 in which dense bone replaces most of the marrow. Osteocytes are prominent. Cortical bone is at the lower edge.  $\times 295$

Fig 10—Femur of a mouse seven months after injection of 0.03 microcurie of plutonium per gram of body weight. It shows a characteristic picture of bone overgrowth in the shaft, with dense bone, the cells and lamellae of which are irregular in arrangement, and with some new, fibrous bone.  $\times 295$



Fig 11—Femur of a mouse seven months after injection of 0.03 microcurie of plutonium per gram of body weight. Note the thick, atypical trabeculae of bone extending into the marrow from the shaft and compare this area with figure 12  $\times 309$

Fig 12—Marrow near the area in figure 11. The cells in the new bone are numerous, large and polymorphous, and are irregularly arranged in sparse, pale-staining matrix. This area is suggestive of early sarcoma  $\times 309$

endosteal surface of the shaft and thickening of the cortical bone vary from specimen to specimen, in several the overgrowth being so extensive as to obliterate the marrow cavity almost completely in places

Nine months after treatment the epiphysis is filled with bone. The metaphysis varies from an increase in thickness of the trabeculae in one specimen to a lacy network filling the metaphysis in another. The shaft bone, however, shows less change than is seen in some specimens at earlier intervals.

The longest interval between treatment and autopsy in this group of animals was eleven months. After this length of time there is a great overgrowth of bone in epiphysis and metaphysis. In one femur the metaphysial and epiphysial bone is continuous, causing an interruption of the cartilage plate. In another specimen, on the other hand, there is hemopoietic marrow on the metaphysial side of the cartilage plate, but below this is a thick band of bone extending completely across the marrow cavity. There are varying degrees of thickening of the shaft bone, but, on the whole, they are not great.

*Vertebrae*—Two months after injection of 0.03 microcurie of plutonium per gram the vertebrae show slight changes, although a number of osteocytes are dead or degenerating (beginning karyolysis) and some lacunas are empty. There is a suggestion of new bone formation. Thin, blue-staining strands of osseous material border some trabeculae of the more compact, preformed bone. At this time the vertebral marrow is extremely depleted and gelatinous.

Later intervals—from three through seven months—show a progressive thickening and fusion of the trabeculae of bone near the cartilage plate and their extension into the marrow cavity, sometimes from the sides as well. This bone is frequently irregular, with its lamellas running in all directions. The proportion of dead bone in this overgrowth varies from specimen to specimen, but in general increases with time, and by seven months much of it near the cartilage is dead. At every stage layers of new, blue-staining bone surround many of the trabeculae. In some of the areas of new bone the nuclei of the osteocytes are swollen, and in others, beginning to degenerate. At one spot in the marrow close to a trabecula there is a small collection of irregular spindle cells, which differ in appearance from typical reticular cells or fibroblasts.

The marrow continues to be mainly gelatinous, although hemopoiesis, beginning at three months, first as sparsely scattered cells or small foci, later increases until whole vertebrae are hemopoietic, although neighboring ones are still completely depleted and gelatinous. From four months on, scattered through the marrow of some specimens are varying amounts of an abnormal homogenous ground substance (like that described in some of the radium-treated mice), sometimes vacuolated, and usually located near an area of hemopoiesis.

From three to seven months after the lower dose of plutonium, 0.003 microcurie per gram, the vertebrae appear much like untreated controls, except for a few specimens in which there is some overgrowth of bone, but there is no correlation between osteosclerosis and length of time after injection, as with the higher dose. In general, new bone is absent, and the marrow is hemopoietic. There are signs of radiation damage, as vacuolated osteoblasts in one instance at seven months, but no striking changes from the normal have appeared.

At later intervals, however, other pathologic changes are seen. Early leukemic changes occur in one specimen at nine months and in another at eleven. In both the bone appears normal. Another specimen has a questionable infarct. And in still another, otherwise normal, one half of the body of vertebra is filled with small trabeculae of normal, pink-staining new bone.

## COMMENT

Similarities and differences in the effects of plutonium and radium on the bones of mice are shown in our histologic material. Both elements produce a progressive overgrowth of bone in epiphysis, metaphysis and shaft. Much of the new bone developing after injection of either radium or plutonium is irregular in arrangement and staining properties, and much of it becomes devitalized. In both instances the newest bone is usually fibrous and is basophilic in decalcified preparations. With the doses used, the new osseous tissue which appears after plutonium tends to be in relatively broad trabeculae of fairly dense bone, irregular in arrangement and thickness of lamellas, while, on the whole, much more fibrous, atypical, acellular bone appears after radium. These variations probably reflect differences in the intensity of ionization and the range of alpha and beta particles. As could be expected from previous experience with these materials, the longer-ranged beta particles, together with the alpha particles coming from the disintegrations of the radium series, destroyed more osteocytes and produced more extensive gelatinous marrow than the short-ranged alpha particles from plutonium. In general, our findings substantiate those of Heller<sup>12</sup> with larger doses of these elements, she characterized the overgrowth of atypical bone after plutonium as apparently being closer to a normal repair process than the dense fibrous bone resulting from injection of radium.

In comparing the effects of plutonium and radium in our experiment, it must be borne in mind that, of the doses administered, only the high dose of plutonium and the low dose of radium were the same in microcuries, and also that the mode of injection was not the same for the two.

Since the changes produced in the bones of our mice are essentially radiation effects, it is necessary to compare the sites of deposition of the plutonium or radium with the sites of greatest damage to the bone as demonstrated histologically. Some bones of the mice of our series were fixed in alcohol for autoradiographs, but we were unable to cut sections of these bones without decalcification because of the great amount of dense new bone in them, so that we do not have autoradiographs of this material that can be compared directly with our decalcified sections. However, we have studied autoradiographs of other mice, similarly treated with radium or plutonium, and examined after shorter intervals than those of the present series (see Heller<sup>12</sup>). On the whole, there is a close correlation between the site of bone changes and the site of radioactivity. The metaphysis, where the overgrowth of bone begins and where the damage to the preexisting bone is greatest, is also the region of heaviest deposition of the radioactive material.

The slower development of osteosclerosis in the epiphysis and diaphysis agrees with the lower concentration of radium or plutonium in these regions in the autoradiographs (see also Calhoun and Aub<sup>14</sup>) Studying the effects of radium and plutonium within the first days and weeks after injection, Heller observed that after radium the overgrowth occurred first in the secondary spongiosa, whereas after plutonium it appeared first in the primary spongiosa In the earliest stages reported here, after injection of both elements, both primary and secondary spongiosa are already involved

In view of the fact that plutonium and radium are deposited on both the endosteal and the periosteal surface of the shaft, although to a lesser extent on the latter, it is interesting that in our sections the overgrowth of bone occurred invariably as an endostosis

In some respects the adult mouse is a more satisfactory experimental animal than the rat, since its bones have practically ceased growing and the pathologic changes are not complicated by the reconstruction of bone due to continued growth

It is difficult to determine the significance of the bone changes described here It is obvious that large amounts of atypical bone are formed, but we find it impossible to classify this bone with precision It is an atypical bone unlike that produced in callus formation or in benign osteoma or frank sarcoma In the mice given radium much of the old and new bone becomes devitalized but shows no definite evidence of resorption, while in the animals given plutonium somewhat less of the new bone is devitalized This new and devitalized bone thus falls within the scope of Ewing's "radiation osteitis" There is, however, no single typical picture of radiation osteitis (Slaughter<sup>15</sup>) As Regaud,<sup>1</sup> Ewing,<sup>2</sup> Phemister<sup>3</sup> and others have pointed out, bone devitalized by irradiation is absorbed slowly if at all In all of our material, we are evidently dealing with earlier stages in the destructive process than any described by Martland in the radium dial painters

In the doses used here, despite the devitalization of large amounts of bone, pathologic fractures did not occur, although many have been observed in rats treated with strontium<sup>89</sup> (Heller<sup>12</sup>, Brues, Lisco, Finkel and France<sup>16</sup>)

In only 2 of about 100 animals were the proliferative bone changes (in femurs in both) suggestive of early sarcoma Here there was neither the atypical fibrous bone characteristic of radium poisoning nor

14 Calhoun, J A, and Aub, J C J Clin Investigation 16 664, 1937

15 Slaughter, D P Am J Roentgenol 48 201, 1942

16 Brues, A M, Lisco, H, Finkel, M P, and France, H O Late Effects of Single and Repeated Injections of Sr<sup>89</sup>, National Nuclear Energy Series (Manhattan Project Technical Section), to be published

the broad band of dense bone so prominent after plutonium. On the contrary, in both of the questionable areas there is only a small amount of osseous tissue, very cellular, with many atypical cells, and containing little interstitial substance. These areas are so small that, although invading adjacent bone marrow, the proliferated cells have not extended to other tissues. Obviously, if they had reached the stage of metastasis, the tumors could not be called early.

It is possible, to be sure, that these growths are not sarcoma, but are exceedingly rapidly forming bits of bone of a different type, seen in only 2 per cent of the bones, all of which had several other types of atypical bone. But since 1 of them (at the seven month interval after plutonium) was seen at a time when early sarcoma is not uncommon in similarly treated animals, and since a large percentage of the remaining animals of these series, allowed to go on to spontaneous death, presented sarcoma (Brues, Lisco and Finkel<sup>13</sup>) and especially in view of the cellularity of these two areas, we are inclined to believe that they probably show the start of a sarcomatous change. Further, many observers reporting in the literature, including Sabin, Doan and Forkner<sup>9</sup> and Dunlap, Aub, Evans and Harris,<sup>8</sup> have described osteogenic sarcomas as occurring frequently after radium treatment, though usually after longer intervals than ours. The descriptions of these later stages of fully developed sarcoma do not help in diagnosing our suspicious material. The changes in the latter are of especial interest, since the events occurring during the long latent period prior to induced cancerous change are little understood.

There is apparently an increase in early leukemias in our animals as compared with controls killed at the same time. Because of the limited number of animals, it is difficult to determine whether this is a significant increase. However, Brues, Lisco and Finkel<sup>13</sup> have observed an increase in incidence of lymphoma and leukemia in CF1 mice treated with plutonium, an increase which occurs also in mice of this strain treated by roentgen irradiation of the whole body.

#### SUMMARY

Radium when injected into mice intraperitoneally at dose levels of 0.3 and 0.03 microcurie per gram of body weight, and plutonium given intravenously at levels of 0.03 and 0.003 microcurie per gram produce an overgrowth of bone in the femurs and the vertebrae. In the femur this overgrowth begins in the distal metaphysis and is more extensive there than in the epiphysis or the shaft. This atypical bone, surrounded by gelatinous marrow, fills the metaphysis and encroaches on the marrow of the shaft, which is also invaded by new

bone from the endosteum, and in the extreme cases the two processes combine in some areas to obliterate the marrow

The bone formed after radium treatment is atypical and fibrous, and much of it becomes devitalized. The cortical bone of the shaft also contains many empty lacunas. After plutonium, the newest bone is also fibrous, but the greatly thickened trabeculae of spongy bone become progressively more compact, although the extremely irregular arrangement of lamellas and osteocytes distinguishes them from normal bone. Empty lacunas are fewer than after radium.

Early changes of an atypical proliferative nature were seen in a three month specimen after radium and in a seven month specimen after plutonium, and may represent early sarcoma or a predisposing state.

Resorption of the necrotic bone does not seem to have occurred.

Infarction of the marrow was seen in 2 of the radium-treated and 1 of the plutonium-treated animals.

The changes in the vertebrae are essentially like those in the femurs.



# Case Reports

## BENIGN MUCOEPIDERMOID TUMOR

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AND

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IN 1945 Stewart, Foote and Becker<sup>1</sup> in reviewing 700 cases of tumors of the major and minor salivary glands were able to separate 45 cases in which the tumors had features sufficiently characteristic to be designated as mucoepidermoid. This descriptive term was applied because both mucoid and epidermoid elements were constant features. In all cases in the group the mucoepidermoid neoplasm took a mucicarmine stain and presented characteristics differing from those of other types of salivary tumors. They found that these tumors fell into two histologic types, benign and cancerous. Of the 45 reported, 26 were placed in the benign category. The benign lesions were equally distributed as to sex, and 58 per cent of the patients had an onset of symptoms before 40 years of age. The parotid gland was the most common site, and the lesion usually appeared as a painless swelling with no fixation to the overlying skin. Grossly the tumors were ovoid, circumscribed, not encapsulated, firm, cystic and varicolored. Forty-two per cent of the lesions were recurrent when first seen. They assumed that these tumors arose from the larger and intermediate ducts which normally are composed of basal, intermediate and columnar cells. The epidermoid and mucous cells apparently result from metaplasia of the cells normally present.

Bernier<sup>2</sup> reported 2 mucoepidermoid tumors, one of which was cancerous and the other benign. This report was preliminary to a more complete survey of salivary gland tumors being done at the Army Institute of Pathology.

The 2 cases to be reported are instances of histologically benign mucoepidermoid tumor.

### REPORT OF CASES

CASE 1—A K, a 19 year old white man, was admitted to the hospital complaining of a painless lump of three years' duration located immediately below the right ear. It had steadily increased in size, and occasionally the overlying skin had become inflamed and painful. Examination revealed a slightly tender, freely movable, multinodular mass located immediately below the right ear. The overlying skin was slightly inflamed. A needle was inserted into the mass and several cubic centimeters of yellow opaque material was aspirated. The overlying skin and the tumor were excised. The lesion extended down to, but did not

1 Stewart, F. W., Foote, F. W., and Becker, W. F. *Ann Surg* 122:820, 1945.

2 Bernier, J. L. *J Oral Surg* 4:153, 1946.

involve the parotid parenchyma. It was ruptured during removal, and a small quantity of mucoid material was liberated from several small cysts.

The specimen consisted of a segment of skin measuring 4.5 by 1 cm to which was attached a mass of subcutaneous tissue measuring 6 by 3 by 3 cm. The cut surface revealed immediately beneath the skin a dark reddish brown multicystic area, measuring 4 by 2 by 2 cm, partially surrounded by soft yellow adipose tissue. The lesion was circumscribed but not encapsulated. A single lymph node was present in the adipose tissue.

Three and one-half months after the initial operation, the patient returned to the hospital complaining of a painless nodule at the superior end of the previous

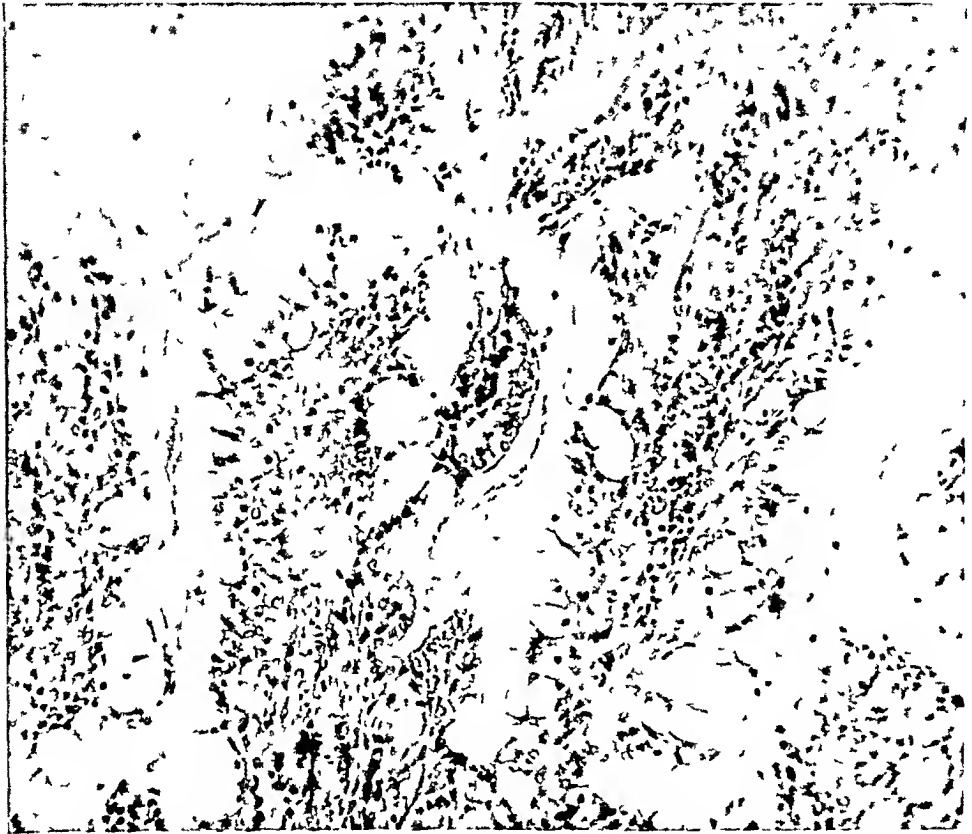


Fig 1 (case 1)—Section showing mucous cells. Hematoxylin and eosin,  $\times 125$ .

operative scar. Examination revealed a firm, freely movable nodule, measuring 3 cm, located beneath the scar. This nodule was removed, and a specimen was also taken from the depths of the operative wound. Grossly the tissue was separated into two portions as removed. The nodule was 2.5 cm in diameter, firm, yellow-gray, not encapsulated and covered on one surface by a small segment of skin. The cut surface of the nodule was composed of two firm multicystic areas with reddish brown centers. The second specimen consisted of three small segments of soft, pinkish gray tissue. All specimens were fixed in formaldehyde solution, sectioned and stained with hematoxylin and eosin and Mayer's mucicarmine.

Microscopic sections of the original tissue revealed a fibrous stroma containing many varying-sized epithelium-lined cysts filled with an eosinophilic staining mucoid substance. The lining cells were flat to columnar and were eosinophilic. In many instances the cell membranes were indistinct. The nuclei varied in size and shape, but no mitotic activity was observed. All types of ductal cells were present, but mucous and epidermoid cells predominated. The mucous cells formed the superficial layer of many of the cyst linings, some of which had a papillary arrangement. A few mucous cells were found deep within the lining, and in some areas small intraepithelial cysts were formed. The basal and epidermoid cells



Fig 2 (case 1) —Section of cyst wall formed by epidermoid cells. Hematoxylin and eosin,  $\times 125$

formed the major portion of the remaining cysts and also showed papillary infoldings. The basement membrane of the cyst linings was intact, although in a few instances mucus had been extravasated into the stroma. There was an associated infiltration of lymphocytes and plasma cells in these areas. Surrounding many of the cysts were large lymphoid follicles. A few intermediate ducts were seen with adjacent distended ducts and mucus-filled cysts of varying size. An occasional plug of epidermoid cells was scattered about in the stroma. No pearl formation or squamous cells were found. A section of skin revealed an area of subcutaneous granulation tissue and scar formation containing several large zones of clear histiocytic cells. Section of the lymph node showed reactive hyperplasia. The

mucous cells and cyst contents took a mucicarmine stain. The structure of the tissues removed at the second operation was similar to that of the original specimen except for an increased number of epidermoid plugs. The tumor involved the margins of the smaller piece of tissue removed at the second operation.

CASE 2—Z N, a 30 year old white woman, complained of a mass of two months' duration located below the right ear. It had slowly increased in size but had not been painful. Examination showed a small, firm, rounded, freely movable, slightly tender nodule just below the right ear. At operation, the tumor was found in the substance of the parotid gland. It was well circumscribed and measured about 7 mm in diameter. It was firm, not encapsulated, and on section showed several 1 mm-sized white nodules. The tissue was prepared similarly to the previous specimens.

Microscopically, the lesion was partially surrounded by normal parotid tissue. It was composed of several cystic areas lined by basal, epidermoid and mucous cells, the mucous type predominating. The cysts contained mucus, and in some areas this had been extravasated into the surrounding tissue. There was an associated infiltration of lymphocytes and plasma cells. The mucous cells and the cyst contents took a mucicarmine stain.

#### COMMENT

The 2 cases described are similar to those previously reported. There has been a recurrence of the lesion in case 1, indicating inadequate removal at the first operation. As pointed out by Stewart and associates,<sup>1</sup> recurrence may be due to outlying hyperplastic foci somewhat removed from the main lesion. A second recurrence may be anticipated in view of the marginal involvement of the tissue removed at the second operation. There has been no recurrence in case 2 after four years. In the previously reported cases, no reference was made to the occurrence of lymphoid tissue in these tumors. In case 1 this was a prominent feature but it could not be established whether this was an integral part of the tumor or incident to extravasation of mucus. The probable ductal origin of these tumors is borne out by the presence of ductal structures in various stages of cystic formation and the resemblance of the cyst epithelium in certain areas to the normal ductal cells.

## Books Received

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PRACTICAL ASPECTS OF THYROID DISEASE By George Crile Jr, M D, Department of Surgery, Cleveland Clinic Cloth Pp 355, with 101 figures Price \$6 Philadelphia and London W B Saunders Company, 1949

This interesting book, based on the author's personal experience of over a thousand cases of thyroid disease, is written for surgeons and internists. It seeks to acquaint each with the domain of the other and to discuss the newer forms of therapy (thiouracil and its derivatives, and radioiodine), which are changing the management of thyroid diseases. Physiology and pathology are briefly discussed in relation to clinical problems. Most of the book is devoted to hyperthyroidism. Endemic goiter, malignant tumors, thyroiditis and congenital abnormalities are mentioned briefly but adequately. On the whole, the views expressed are conventional. The pathologic classification of thyroid tumors used is that of Graham. This book is heartily recommended to those interested in the clinical aspects of thyroid diseases.

HINDU MEDICINE. By Henry R Zimmer, Ph D, late visiting lecturer in philosophy at Columbia University, formerly professor of sanskrit at the University of Heidelberg. With a foreword and preface by Ludwig Edelstein, Ph D. Cloth Pp 203 Price \$4 Baltimore The Johns Hopkins Press, 1948

The present volume has been compiled from the seventh course of lectures of the Hideyo Noguchi Lectureship delivered in 1940 at the Johns Hopkins Institute of the History of Medicine by the late Professor Zimmer. His study of Hindu medicine was part of a larger analysis of Hindu culture. The two lectures take up such medical subjects as the relation of Hindu to Greek medicine, psychotherapy, medicine in the Vedic tradition, and the development, extent and transmission of Hindu, medical and related knowledge.

Here are traced ideas as they emerge from mythology and become modified by accumulated racial wisdom. A groping for safe rules of health is apparent—a search which was foredoomed to failure because of the state of knowledge then extant. In a subtropical country with no knowledge of bacteriology medicine is greatly handicapped, even the study of anatomy becomes difficult. This is a scholarly and interesting book which can be recommended.

## PULMONARY ADENOMATOSIS OF MAN

A Review of the Literature and a Report of Nine Cases

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THE pulmonary adenomatoses, or so-called alveolar cell tumors of the human lung, constitute a unique histologic group among the primary pulmonary neoplasms. There is general agreement that the majority of primary tumors of the lung arise from the bronchial respiratory epithelium. There is little unanimity of opinion, however, regarding the histogenesis of the adenomatoses, although most investigators concur that the site of origin is independent of the bronchi.

There are few conditions which offer as great a challenge to investigators as pulmonary adenomatosis, for whether or not it is a true neoplasm is still a matter of controversy. The etiologic concept is obscure and is complicated by conflicting claims of proponents of diverse theories. A unique opportunity to gain an insight into these problems was made possible by studying the relatively large series at the Army Institute of Pathology. Approximately 900 pulmonary neoplasms were screened to discover all cases of pulmonary adenomatosis which might have been filed under a designation other than those usually employed. Nine cases were found which satisfactorily fulfilled the criteria established for the purposes of this study.

It is the object of this paper (1) to review cases not included in those collected by Neuburger and Geever<sup>1</sup> in 1942, (2) to present and discuss 9 cases from the files of the Army Institute of Pathology, (3) to examine theories concerning histogenesis and cause and (4) to discuss the question of whether pulmonary adenomatosis is related to carcinoma of the lung.

### CRITERIA

It is unfortunate that precise criteria have not been fixed to limit the use of the term "pulmonary adenomatosis." Therefore, it is proposed that it be reserved for tumors which fulfil the following criteria: (1) alveolar cellular proliferation, characterized by the appearance of tall columnar mucus-producing cells, (2) absence of an intrinsic tumor of the bronchial tree and (3) absence of primary adenocarcinoma of any

From the Army Institute of Pathology, Washington, D. C.

<sup>1</sup> Neuburger, K. T., and Geever, E. F., Arch. Path. 33:551, 1942.

other part of the body. Those tumors which had involved regional lymph nodes or given rise to distant metastases but which otherwise conformed to the specified pattern were also included and were regarded as cancerous variations of pulmonary adenomatosis. Investigation has disclosed that adenomatosis has often been classified as an unusual example of bronchogenic carcinoma, therefore, it is not improbable that neoplasms thus diagnosed may have been included in material from which statistics pertaining to adenocarcinoma of the lung have been derived.

#### NOMENCLATURE

Some concept of the difficulties inherent in searching the literature for accounts of these tumors can be gained by considering the diversity of names selected. Judging from the names, one perceives that some authors considered this neoplasm benign, others, cancerous. There is still another group who have been noncommittal regarding origin or prognosis and have chosen unprejudicial designations. Among the many appellations are "adenoma-like tumor,"<sup>2</sup> "pulmonary adenomatosis,"<sup>3</sup> "primary multiple alveolar cell tumor,"<sup>4</sup> "papillary gelatinous adenocarcinoma,"<sup>5</sup> "alveolar cell cancer,"<sup>6</sup> "multicentric alveolar cell carcinoma,"<sup>7</sup> "carcinomatoides alveogenica multicentrica,"<sup>8</sup> "diffuse primary alveolar epithelial carcinoma,"<sup>9</sup> "mucocellular papillary adenocarcinoma,"<sup>10</sup> "malignant adenomatosis,"<sup>11</sup> "carcinosis,"<sup>12</sup> and "diffuse epithelial hyperplasia."<sup>13</sup> Recently "alveolar cell tumor" has become a popular choice,<sup>14</sup> but unfortunately it is often misinterpreted to mean that the tumor is derived from "alveolar lining cells" or has the morphologic configuration of alveoli, although the authors did not intend either implication.

2 Helly, K. *Ztschr f Heilk* **28** 105, 1907

3 Richardson, G. O. *J Path & Bact* **51** 297, 1940

4 Neubuerger, K. T. *J Thoracic Surg* **10** 557, 1941

5 Briese. *Frankfurt Ztschr f Path* **23** 48, 1920

6 Sweany, H. C. *Arch Path* **19** 203, 1935

7 Reuss, H. *Ueber zwei Falle multicentrisch entstandener Lungenkrebse*, Inaug. Dissert. Hamburg, 1934

8 Casilli, A. R., and White, H. J. *Am J Clin Path* **10** 623, 1940

9 Godel, A. *Frankfurt Ztschr f Path* **29** 392, 1923. Weismann, S. *ibid* **47** 534, 1935

10 Osserman, K. L., and Neuhof, H. *J Thoracic Surg* **15** 272, 1946

11 Dacie, J. V., and Hoyle, C. *Brit J Tuberc* **36** 158, 1942

12 Bonne, C. *Am J Cancer* **35** 491, 1939

13 Bell, E. T. *Am J Path* **19** 901, 1943

14 (a) Geever, E. F., Neubuerger, K. T., and Davis, C. L. *Am J Path* **19** 913, 1943. (b) Geever, E. F., Carter, H. R., Neubuerger, K. T., and Schmidt, E. A. *Radiology* **44** 319, 1945. (c) Neubuerger<sup>4</sup>

One hesitates to coin a new term to designate any condition in the nomenclature of which so much confusion already exists. However, "adenomatosis" is a proper designation for tumors which fulfil the three criteria enumerated, and "cancerous adenomatosis," for those which metastasize in addition. The descriptive phrase "epithelization of the alveolar walls" is also regarded as inappropriate, for many authorities deny that the adult pulmonary alveoli are lined by cells of epithelial origin. Therefore, "investment of alveoli" has been substituted, deliberately evading any speculation concerning the origin of the cells which line the alveoli in abnormal conditions such as pulmonary adenomatosis.

#### MATERIAL AND METHODS

The material on which this study was based has been derived from 9 cases collected in the files of the Army Institute of Pathology from military and civilian sources. Four additional cases were rejected because the data were incomplete or the postmortem observations controversial.

In every case in which the material was sufficient the following stains were used: hematoxylin and eosin, Masson's trichrome, Weigert's elastic and mucicarmine. In a few the Brown-Brenn and Giemsa stains were used. All sections were cut from formaldehyde-fixed tissues and paraffin blocks, however, in 1 instance additional celloidin (pyroxylin) sections were made for comparative purposes.

The Masson and elastic stains adequately demonstrated that the normal components were present in the alveolar walls. The Masson stain was superior to hematoxylin and eosin for the study of the structure of the investing cells. The mucicarmine stain revealed that the cells were secretory in several instances, but it is believed that when mucin was not seen, the tissue may have been washed free of it by long immersion in fixative. No bacteria were noted with the special stains. In our experience paraffin sections maintain the delicate cellular investment of the alveolar walls with no more distortion than is seen in sections made from celloidin-impregnated tissue.

#### CASES FROM THE ARMY INSTITUTE OF PATHOLOGY

**CASE 1** (contributed by Arturo R. Casali, M.D., Elizabeth, N.J.)—A white man aged 59 was admitted to a hospital with the history of sudden pain in the chest followed by increasingly severe dyspnea. Roentgenograms revealed discrete, nodular infiltration throughout both lung fields, and the clinical impression was pulmonary tuberculosis. There was progressive loss of weight and weakness, and the patient died approximately eight months after the onset of symptoms.

At autopsy, the right lung was firmly adherent throughout its entire posterolateral aspect. There were no adhesions of the left lung. The pleural cavity contained about 100 cc of clear yellow fluid. The cut surface was studded with nodules, many of which were confluent (fig. 1). No metastases were noted.

**Microscopic Observations**—The alveolar spaces were lined by cuboidal to columnar cells (figs. 2 and 3). The nuclei were situated close to the cell bases, the nuclear membrane was sharply defined and the nucleolus was small, round, eccentrically placed and hyperchromatic. The chromatin was finely distributed, and no mitotic figures were present. The cytoplasm was eosinophilic and



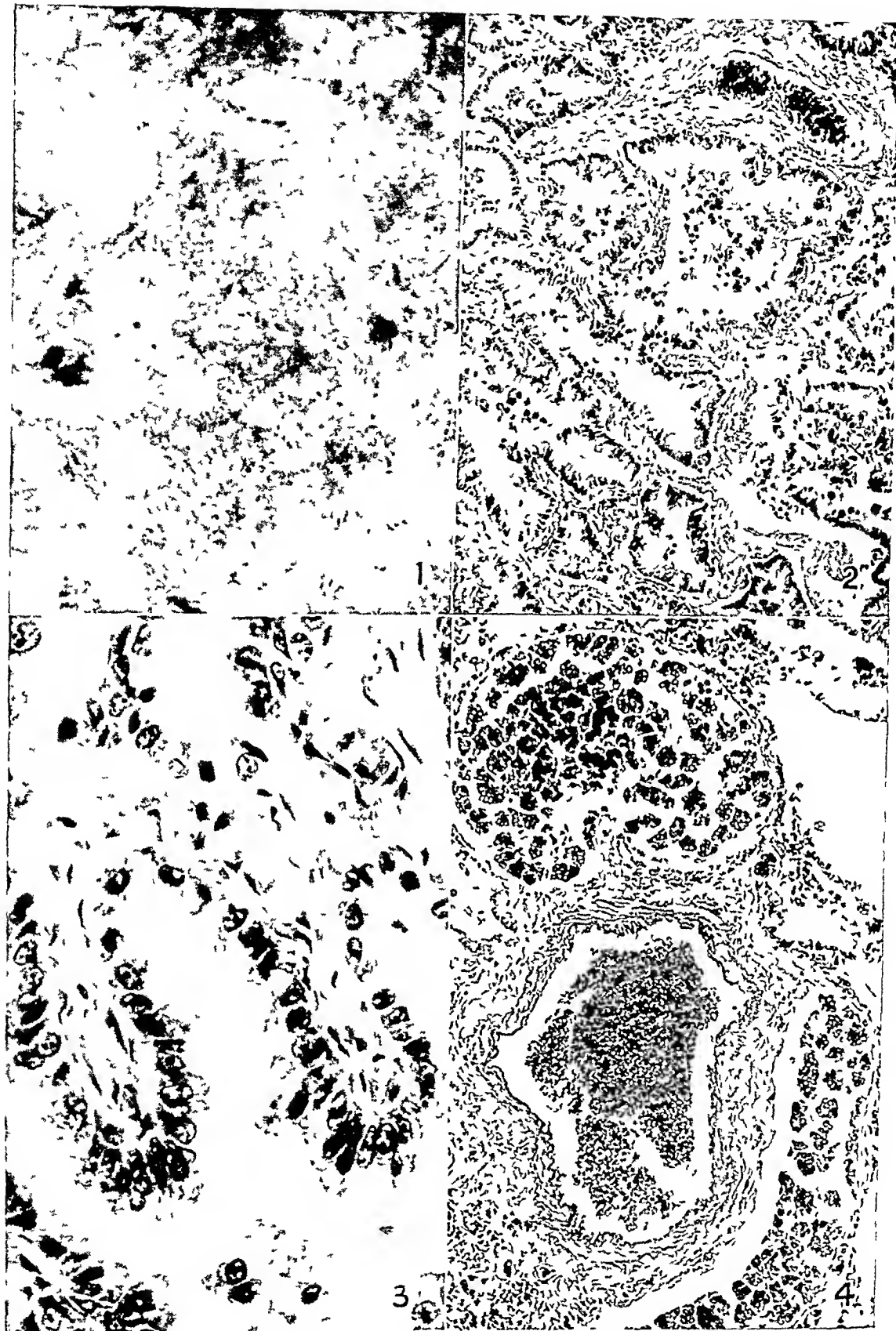


Fig 1—Cut surface of the right lung. There are numerous discrete and coalescing nodules. Army Institute of Pathology negative 99355

Fig 2—Alveolar walls are slightly thickened and lined by cuboidal and columnar cells. There are occasional papillae and foci of desquamated lining cells.  $\times 145$ . Army Institute of Pathology negative 85106

Fig 3—Detail of investing cells. Note formation of papillae.  $\times 658$ . Army Institute of Pathology negative 85099

Fig 4—Papillary proliferation of atypical cells in perivascular lymphatic channels.  $\times 130$ . Army Institute of Pathology negative 85107

prominently vacuolated. Numerous delicate papillae partially filled the alveolar spaces. There were also many rounded individual cells, which were phagocytic, and rare polymorphonuclear leukocytes in the alveoli. The alveolar walls were intact, and there was no evidence of necrosis.

The medium-sized branches of the pulmonary artery demonstrated reduplication of the internal elastic lamina. The peribronchial and perivascular lymphatic channels were lined by cells similar to those occupying the alveolar walls (fig 4).

CASE 2—A white man, aged 40, when first seen complained of pain in the chest and cough. His temperature was 102 F, and acute bronchitis was diagnosed. Four months later he was seen again. Coughing had increased, and he expectorated small quantities of thick white phlegm. Roentgenograms of the chest revealed consolidation of the lower lobe of the right lung. Sputum became copious and was not blood streaked. Seven months after the initial symptoms the patient was producing from 1½ to 2 cups of mucoid sputum daily, in which no blood was noted. He became progressively weaker, with marked dyspnea on exertion, and died approximately eight months after he first was admitted to the hospital.

At autopsy the lungs were voluminous, filling the pleural spaces. There were bilateral adhesions with no loculated fluid. The right lung weighed 1,340 Gm. The lower lobe was firm, and the cut surface was studded with soft white nodules varying in size from bare visibility to a diameter of 2.0 cm. The left lung weighed 850 Gm and was crepitant throughout, and there was no gross evidence of tumor. No necrosis or evidence of metastasis was noted.

*Microscopic Observations*—The alveolar spaces were lined by a single row of nonciliated tall cylindric cells (figs 5 and 6). The cells formed a striking pattern with their orderly array, lack of hyperchromatism, mitotic figures and lack of pleomorphism. The numerous papillary processes had delicate central stalks containing tiny capillary vessels. A bright red nucleolus, slightly eccentrically placed, was visible with Masson's stain. There were many mucin-filled vacuoles in the cytoplasm. The alveolar walls had been ruptured by massive intra-alveolar cellular proliferation. The normal mural components could be identified with special stains. No metastases were noted.

CASE 3—A 31 year old white man had undergone two episodes of "flu," one in 1941, another in 1942, spending a week in the hospital on each occasion. Two months after the second episode he noted dyspnea and cough on slight exertion. The roentgenologist's report described scattered areas of exudative infiltration throughout the entire right lung and parts of the left, and the diagnosis was "far advanced tuberculosis." Repeated examination of sputum failed to reveal acid-fast organisms or fungi. The patient's course continued downhill, with progressively severe dyspnea, orthopnea, weakness and cough. Among the diagnoses considered were pulmonary tuberculosis, Boeck's sarcoid and histoplasmosis. The patient died three months after the onset of symptoms.

At autopsy, the left lung weighed 700 Gm. It cut with what was described as a "gritty resistance," disclosing numerous discrete and confluent, poorly circumscribed red-gray nodules. The right lung resembled the left. No metastases were noted.

*Microscopic Observations*—Although extensive areas of the pulmonary parenchyma had been obliterated by fibrosis, there were many islands of surviving alveoli. The alveolar walls were lined with a single layer of tall cylindric cells, many of which were ciliated (fig 7).



Fig 5—Alveolar walls are lined by tall cylndric cells Papillary processes are frequent  $\times 100$  Army Institute of Pathology negative 85110

Fig 6—Detail of nonciliated columnar investing cells Note intracytoplasmic vacuoles which contain mucin  $\times 500$  Army Institute of Pathology negative 85100

Fig 7—Thickened alveolar walls lined by ciliated cylndric cells  $\times 300$  Army Institute of Pathology negative 85098

The cytoplasm contained secretory granules, and the alveolar spaces were filled with mucoid material in which there were many mononuclear cells and lymphocytes, moderate numbers of polymorphonuclear leukocytes and occasional multinucleated monster giant cells. There was marked peribronchiolar inflammatory cell infiltration. No evidence of metastasis was noted.

CASE 4 (contributed by David M. Grayzel, M.D., Brooklyn)—The patient was a 43 year old white man. During a fluoroscopic examination he was found to have a "spot" on the right lung. He was treated twelve times with high voltage roentgen rays, presumably for carcinoma of the lung. He had had a cough for an undetermined time, which became severe ten days before he was admitted to the hospital. Roentgenograms of the chest revealed an opacity obscuring the right lower lung field. Pneumonectomy was performed on the right side. Six months later the patient died with massive pleural effusion. Permission for autopsy was not granted, but there was no clinical evidence of recurrence of tumor.

The right lung, removed at operation, weighed 1,240 Gm. The lower lobe was voluminous, grayish pink and white, and of a rubbery consistency. The cut surface was studded with closely approximated grayish yellow nodules (fig. 8). A description of the left lung is unavailable.

*Microscopic Observations*—The alveoli were lined by nonciliated tall cylindrical cells, and the spaces were almost solidly filled with papillary processes. The nuclei were situated at the bases of some cells and at the apexes of others. Those cells having the nuclei situated at the free ends were distinctly club shaped. There was abundant cytoplasm with various-sized vacuoles containing material which stained red with mucicarmine. In many alveoli a delicate wrinkled membrane lay in close approximation to the alveolar wall.

The alveolar walls were intact and acted as a supporting stroma for the lining cells. The intramural capillaries were engorged and contained many polymorphonuclear leukocytes. Several areas of lung parenchyma contained sheets of polymorphonuclear leukocytes associated with microabscesses.

The bronchioles were surrounded by alveoli lined with investing cells, however, the bronchiolar epithelium consisted of normal cells. Dr. Nathan Chandler Foot commented on these sections as follows: "Bronchogenic adenocarcinoma. Might well have originated in pulmonary adenomatosis."

CASE 5—A 66 year old white man complained of long-standing productive cough, pain in the chest and dyspnea on exertion. There had been hemoptysis for three months prior to hospitalization. A roentgenogram revealed atelectasis of a portion of the lower lobe of the right lung. Pleural fluid was obtained and an examination made for cancer cells and acid-fast organisms, none was found. A bronchoscopic examination revealed moderate secretion from the bronchus of the lower lobe of the right lung and no evidence of tumor. The patient was discharged from the hospital. At home he continued to be troubled with a cough productive of a moderate amount of mucoid material. His condition did not improve, and he suddenly died after approximately fifteen months' illness.

At autopsy there were a few fibrous adhesions and no effusion in the right pleural space. The left pleural space contained 300 cc of clear straw-colored fluid and occasional bandlike adhesions were noted at the apex and the lower lobe of the left lung. The interlobar fissures were obliterated. The right lung weighed 1,287 Gm. The cut surface of the entire lung was gray and consolidated.

except at the periphery, where there were numerous small nodules. The left lung weighed 2,015 Gm. The cut surface was similar to that of the right lung.

The bronchi of both lungs appeared to be normal, and no metastases were noted.

*Microscopic Observations*—There was an increase in the amount of fibrous tissue throughout the bronchial wall, marked peribronchial fibrosis and moderate hyperplasia of the mucous glands, but no evidence of bronchogenic tumor. The alveoli surrounding the bronchus were lined by nonciliated cuboidal and columnar cells (fig 9). The investing cells projected irregularly into the alveolar spaces with bulbous or whiplike processes, often containing several nuclei. There were many mucus-filled cytoplasmic vacuoles. The alveolar walls were delicate and formed a latticework for the neoplastic cells. There were extensive areas of fibrosis, and special stains revealed marked obliterating endarteritis of the medium-sized pulmonary arteries. No metastases were noted.

CASE 6—A white man 35 years of age was observed, by routine roentgenogram of the chest, to have evidence of pulmonary disease. He had had no premonitory signs or symptoms and was completely unaware of the existence of pulmonary disease. Lobectomy of the left lower lobe was performed, and no extension to the adjacent lung or to the regional lymph nodes was noted. The patient's immediate postoperative course was uneventful.

In June 1947, roentgenograms of the chest revealed lesions in the upper and middle lobes of the right lung. A specimen was taken from the upper lobe for biopsy, and Dr. Sidney Farber immediately inoculated laboratory animals, including sheep and guinea pigs, with some of the tissue and planted some in culture mediums. No growth was obtained on culture, and no evidence of disease was noted in the inoculated animals.

Biopsy showed that the material examined consisted of the surface of the lower lobe, which was mottled gray and black and showed no adhesions. On cut surface several nodules measuring up to 2.5 cm were encountered. These were present in the subpleural region, and one was found lateral to the main stem bronchus but did not communicate with it. The surface of the nodules was smooth, glistening, gelatinous, and divided into coarse trabeculations. Dissection of the bronchi revealed no abnormalities, and several hilar lymph nodes appeared to be free of metastases.

*Microscopic Observations*—There was a remarkable investment of the alveolar walls by large, tall columnar cells, which were actively secreting a mucoid material. The alveolar spaces contained this mucoid substance and many desquamated lining cells. In several places the production of mucus was so copious that the alveolar walls had ruptured, allowing pools of mucus to form (fig 10). The pulmonary stroma was intact in all other areas. No metastases were noted in the sections of hilar lymph nodes or peribronchial lymphatic vessels.

CASE 7 (contributed by Theodore L. Bliss,<sup>15</sup> M.D., Akron, Ohio)—A 47-year-old white man first noticed an unproductive cough. He was a chemist and stated that he had long been exposed to sulfur dioxide and other chemical pulmonary irritants. Within four months the cough became productive. During the fifth month of his illness he became febrile and raised copious amounts of thin fluid. Roentgenograms of the chest revealed increased density in the region of the middle lobe of the right lung. Laboratory examinations did not reveal tubercle bacilli or fungi.

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15 Bliss, T. L. J. Thoracic Surg. 6:660, 1936.

On bronchoscopic examination, profuse secretion was seen to well up from the bronchus of the lower lobe of the right lung, which had become narrowed. The quantity of bronchial secretion in a twenty-four hour period was prodigious and was estimated to vary between 1,200 and 1,800 cc. The secretion was watery, never contained blood or pus and had no odor and no taste.

The patient died approximately thirteen months after the onset of symptoms.

At autopsy, the right pleural cavity was obliterated by dense fibrous adhesions, and there were a few easily separated adhesions in the left pleural cavity. On section, the entire right lung was seen to be replaced by grayish white tumor. Approximately one third of the left lung, in the vicinity of the hilus, was replaced by similar neoplastic tissue.

*Microscopic Observations*—The alveolar walls were lined by nonciliated columnar cells, which appeared to be arranged in a pseudostratified fashion. The cytoplasm was strongly eosinophilic, and the nuclei were round to oval and were usually situated near the bases of the cells, however, where the lining cells were irregularly grouped, nuclei were situated near the tips of the cells. The alveolar walls were of normal width and furnished a lattice on which the proliferating cells formed papillary processes. The pulmonary septums were more prominent than normal and contained occasional lymphocytes, plasmacytes and polymorphonuclear leukocytes. No metastases were present in the peribronchial or pleural lymphatic channels.

CASE 8—A 62 year old woman complained of having a feeling of irritation in the posterior part of the pharynx, incapacitating dyspnea on exertion and persistent cough with production of small amounts of clear mucoid material, without hemoptysis.

Bronchoscopic examination revealed an anterior-posterior narrowing of the dorsal branch bronchus of the lower lobe of the right lung, and no endobronchial tumor was seen. Examination of pleural fluid obtained from the right cavity of the chest was reported as "carcinoma cells morphologically suggesting metastatic epithelial origin."

Roentgenograms revealed uniform density of the lower half of the right lung and a fine type of miliary infiltration throughout the upper portion of the right and the entire left lung.

Respiratory distress was extreme and constant. Weakness, cyanosis and dyspnea increased in severity, and the patient died approximately five months after the onset of symptoms.

At autopsy, the pertinent findings were limited to the lungs; no metastases were noted in the regional lymph nodes. The gross description of these lungs is unavailable.

*Microscopic Observations*—The pleura was greatly thickened and fibrous, and scattered through it were small oval spaces resembling lymphatic channels lined by a single layer of large atypical cells. The alveolar walls were also lined by a layer of cells identical to those seen in the pleura. The alveolar walls were slightly wider than normal, however, special stains failed to demonstrate any fibrosis, and the elastic and reticular elements were normal. The neoplastic cells were present in the perineural (fig 11), perivascular and peribronchial lymphatic channels. In most places these cells lined the lymphatic walls in a manner identical to that seen in the alveoli, and the lumens contained occasional desquamated cells. The structure of the peribronchial lymph nodes was partially obliterated by these cells. No mitotic figures were noted in the parenchymal or the lymphatic neoplastic cells.





Fig 8—Diffuse involvement of the right lower lobe of the right lung. Note the smooth pleural surface and suggestion of peripheral nodularity on cut section. Army Institute of Pathology negative 98159.

Fig 9—Low power view showing investing alveolar cells. The alveolar walls are thickened. Note the remarkable similarity between this picture and that seen in jagzietke (fig 14). Army Institute of Pathology negative 99404.

Fig 10—Pools of mucus in alveolar spaces. The investing cells have large intracytoplasmic vacuoles.  $\times 390$ . Army Institute of Pathology negative 99399.

Fig 11—Perineural lymphatic vessels lined by cells identical with those investing the alveoli.  $\times 450$ . Army Institute of Pathology negative 100365.

CASE 9—A 42 year old white man was admitted to the hospital, complaining of shortness of breath and pain in the right side of the chest. It was believed that he had a tumor of the right lung, and bronchial biopsy was said to show tumor which probably arose in the gastrointestinal tract. Roentgenograms of the gastrointestinal tract failed to demonstrate any abnormalities. An exploratory thoracotomy revealed collapse of the lower lobe of the right lung and presence of nodules throughout the right leaf of the diaphragm.

The course of the disease was marked by effusion, requiring repeated aspirations of the right pleural cavity, and developing empyema. Cough was moderate, and 2 to 3 ounces (60 to 90 cc) of sputum was produced daily. After a prolonged downhill course, the patient's condition deteriorated rapidly, and he died approximately fifteen months after the onset of symptoms.

At autopsy, the right pleural cavity contained a large quantity of semifluid greenish black material, and the left pleural cavity contained 3,300 cc of thin amber-colored fluid. The cut surfaces of the lower lobes were gray, and dissection of the bronchial tree failed to demonstrate either endobronchial tumor or ulceration. The hilar and mediastinal lymph nodes were grossly invaded by tumor.

Tumor nodules were present over the entire surface of the right leaf of the diaphragm. The liver contained numerous gray nodules, which did not elevate Glisson's capsule and were not umbilicated. The right adrenal gland contained an 8 mm tumor in the medulla. There were also metastases in the periaortic lymph nodes, and several implants were present on the serosa of the gastrointestinal tract.

*Microscopic Observations*—Near the pleural-parenchymal junction numerous lymphatic channels were lined by atypical cells. The alveolar walls had been diffusely invested by similar cells. These cells were large, with eosinophilic granular cytoplasm. The nuclei were regular in size, and no mitotic figures were seen. In some areas the cells were clumped, giving the appearance of pseudogiant cells. Desquamated cells were present in the alveolar spaces, and large cytoplasmic vacuoles were common. The alveolar walls were of normal width, and there were occasional papillary processes.

The peribronchial lymphatic channels were filled with proliferating tumor cells, and the regional lymph nodes had been almost completely replaced by tumor tissue. The microscopic picture of the local and distant metastases (figs 12 and 13) resembled that of the pulmonary parenchyma, and mitotic figures were rare.

#### ANALYSIS OF CASES FROM ARMY INSTITUTE OF PATHOLOGY

A review of the data of the 9 cases from the Army Institute of Pathology shows that the ages of the patients varied from 31 to 66 years, with an average age of 47.2 years. The sex incidence was 8 males to 1 female. The preponderance of males can be explained by the selective nature of the material acquired by the Army Institute of Pathology. All the patients were white.

The duration of the disease was extremely difficult to evaluate, but in 8 cases in which there were symptoms the duration from the onset of the symptoms varied from four to fifteen months. In case 6 there were no symptoms and the lesion was discovered by routine roentgenologic examination (see table 1).



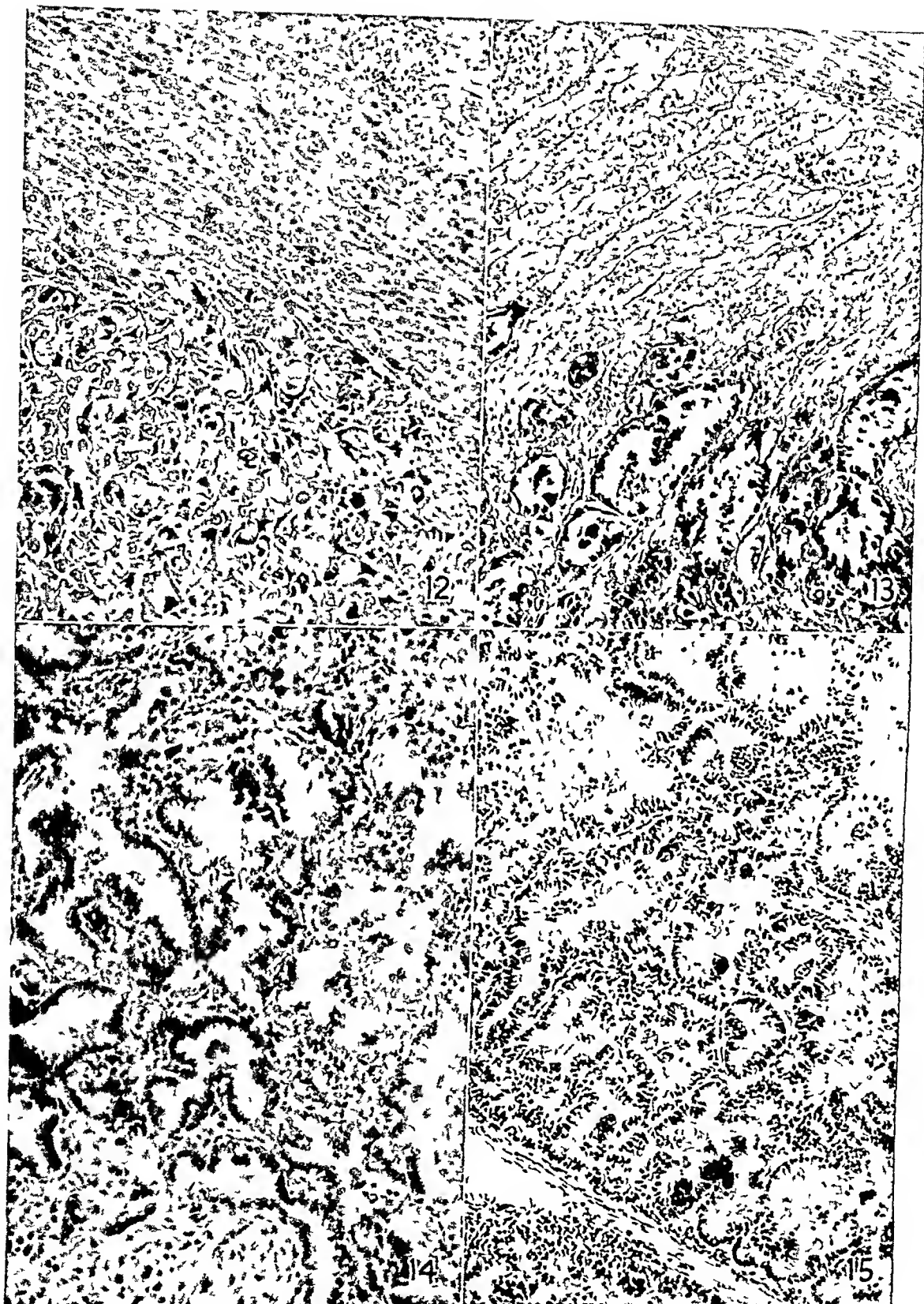


Fig 12—Metastasis in liver The cells show considerable anaplasia, however, mitotic figures are rare  $\times 114$  Army Institute of Pathology negative 100382

Fig 13—Metastasis in adrenal gland Note the similarity of pattern between the metastasis and the growth seen in the lung  $\times 114$  Army Institute of Pathology negative 100381

Fig 14—Jagzietke in sheep Note the similarity between this and human pulmonary adenomatosis (fig 9)  $\times 160$  Army Institute of Pathology negative 99551

Fig 15—Caprine pulmonary adenomatosis  $\times 115$  Army Institute of Pathology negative 100379

(Contributed by C L Davis, DVM)

The history of pulmonary irritants was established in case 7 only, the patient had been exposed to irritating chemical fumes in a fertilizer plant for many years. One patient (case 2) had been a cab and truck driver and may have been exposed to exhaust fumes. In most of the remaining cases a history of repeated bouts of "flu" or colds was given. Only in case 5 was there a history of heavy smoking.

Signs and symptoms in order of frequency were productive cough, fever, dyspnea, weakness, loss of weight, thoracic pain, fatigue, cyanosis, night sweats, pleural effusion and clubbing of the fingers.

The usual clinical impressions in order of frequency were tuberculosis, bronchogenic carcinoma, bronchopneumonia, bronchiectasis, Boeck's sarcoid, histoplasmosis and coccidioidomycosis.

TABLE 1—*Nine Cases of Pulmonary Adenomatosis Discovered by Screening Material at the Army Institute of Pathology*

| Case | Army Institute<br>Pathology<br>Accession | Sex | Age | Race | Location                                   | Source<br>of<br>Material | Metastases  | Dura-<br>tion,<br>Mo. |
|------|--|-----|-----|------|--|--------------------------|---|-----------------------|
| 1    | 58048                                    | M   | 59  | W    | Bilateral                                  | Autopsy                  | Lymphatic channels  | 8                     |
| 2    | 96875                                    | M   | 40  | W    | Right lung                                 | Autopsy                  | None  | 4                     |
| 3    | 107856                                   | W   | 31  | W    | Bilateral                                  | Autopsy                  | None  | 4                     |
| 4    | 132190                                   | M   | 43  | W    | Lower lobe, right lung                     | Pneumonec-<br>tomy       | Rarefaction in left<br>tibia and questionable<br>metastases in mesen-<br>teric nodes            | 12                    |
| 5    | 147459                                   | W   | 66  | W    | Bilateral, esp. lower<br>lobe, right lung  | Autopsy                  | None  | 15                    |
| 6    | 165404                                   | M   | 35  | W    | Lower lobe, left lung                      | Lobectomy                | None  | 2                     |
| 7    | 170824                                   | M   | 47  | W    | Bilateral mostly<br>lower lobe, right lung | Autopsy                  | None  | 13                    |
| 8    | 174836                                   | F   | 62  | W    |  | Autopsy                  | Bronchial lymph nodes   | 5                     |
| 9    | 105690                                   | M   | 42  | W    | Bilateral                                  | Autopsy                  | Metastases in dia-<br>phragm, liver, adrenal<br>gland, periaortic lymph<br>nodes and peritoneum | 15                    |

The pathologic material was obtained from seven autopsies, one pneumonectomy and one lobectomy. The patient on whom pneumonectomy was done died six months after operation but permission for autopsy was not granted. The patient on whom lobectomy was done has survived to the date of this writing.

The distribution of the tumor, whether nodular or diffuse, was not always apparent from the descriptions of the gross specimens and the impression gained was that in most cases the process was actually a coalescence of nodules. There was no necrosis of the tumor. Metastases were noted grossly in only 1 case.

Histologically, these cases fulfilled all the criteria for pulmonary adenomatosis. Metastases were limited to the parenchymal lymphatic

channels in 1 lung (case 1) and to parenchymal lymphatic channels and hilar lymph nodes in 1 (case 8), there was widespread metastasis only in case 9. In the patient on whom pneumonectomy was done, there was a suspicion that metastases were present, but in the absence of autopsy confirmation was not possible.

#### ANALYSIS OF COLLECTED CASES

The data of cases described in the available literature were compiled by Neubuerger and Geever<sup>1</sup> in 1942, and among the tumors were 25 which these authors regarded as unmistakable "alveolar cell tumors." The ages of the patients varied from 20 to 89 years, and the sex ratio was given as approximately 1:1. A review of a majority of these cases has revealed that they fulfil the criteria given for pulmonary adenomatosis.

Twenty-six case reports have appeared in the literature since 1941, and I have added one<sup>16</sup> which had been published prior to 1941 but which Neubuerger and Geever did not include in their review. In table 2 are recorded 12 of these cases of pulmonary adenomatosis in which no metastases were found, and in table 3, 15 cases in which metastases had occurred. The 27 cases in tables 2 and 3 adequately fulfil all criteria previously enumerated. Table 4 lists 3 cases in which the condition described may be considered doubtful pulmonary adenomatosis and which are therefore not included in the following analysis.

Analysis of the 27 acceptable cases reveals that the ages of the patients varied from 17 to 79 years. Ten were between 50 and 59, 5, between 40 and 49, and 5, between 60 and 69. Thus 20 of the tumors (74.1 per cent) occurred in the 40 to 70 year age group. Twelve patients (44.4 per cent) were men and 15 (55.6 per cent) were women. Evidence of invasion or metastasis was present in 15 (table 3), or 55.6 per cent.

A comparison was made of 100 patients with bronchogenic carcinoma of the lung whose records are in the possession of the Army Institute of Pathology and the 27 patients with pulmonary adenomatosis reported in the literature and reviewed here, which revealed that the latter made up a considerably older group. The difference can be explained, however, by the fact that the two sets of cases are samples drawn from two populations known to be different, Army and civilian, respectively. The relative predominance of this condition in the female sex is remarkable when one considers that in series of cases of bronchogenic carcinoma the ratio of men to women varies from 3:1<sup>17</sup> to 10:1<sup>18</sup>. Therefore the

16 Sayago, G. *Prensa med argent* **19** 545, 1932.

17 Arkin, A., and Wagner, D. H. *J A M A* **106** 587, 1936.

18 Grove, J. S., and Kramer, S. E. *Am J M Sc* **171** 250, 1926.

TABLE 2—Cases of Pulmonary Adenomatosis Without Metastases Collected from the Literature

| Author  | Age | Sex | Thoracic Findings   | Authors' Interpretation   |
|---|-----|-----|---|---|
| Sayago, <sup>16</sup> 1932                                  | 33  | M   | No pleural adhesions Both lungs congested, gray hepatization throughout lower lung fields, millary nodules, many confluent, scattered throughout lower lobes<br>Microscopic Alveolar walls invested with mantle of cuboidal and cylindric cells, papillae frequent, no mucus demonstrated   | "Belongs to group of tumors arising from alveoli"   |
| Sims, <sup>24a</sup> 1943                                   | 42  | M   | In each pleural cavity, about 100 cc of serous sanguinous fluid Adhesions present Lungs voluminous, multiple nodules bilaterally, most extensive in right lung<br>Microscopic Alveoli lined by single layer of high cuboidal cells, alveolar walls normal   | "the alveolar exudate and the epithelial hyperplasia are quite like those in the jag ziekte sections"   |
| Bell <sup>13</sup> 1943                                     | 63  | M   | Diffuse consolidation of lobes of both lungs<br>Microscopic Alveoli lined by cuboidal and columnar cells, alveolar walls and pulmonary septums essentially normal   | "There is convincing evidence that the epithelium forms locally and does not grow from bronchi"   |
| Taft and Nickerson, <sup>46d</sup> 1944                     | 62  | M   | Numerous fibrinous adhesions bilaterally Left lung 900 Gm, firm, consolidated, cut surface of upper lobe grayish white Right lung 850 Gm, almost completely consolidated<br>Microscopic Alveolar walls covered by columnar cells and occasional papillae  | "While a definite conclusion cannot be made, it seems probable that the abnormal cells arise from the alveolar lining"                                  |
|   | 79  | F   | Right lung 700 Gm on section lower and middle lobes were gray centrally and dark red peripherally Left lung 420 Gm, on section lower lobe gray, gelatinous<br>Microscopic Alveoli lined by columnar cells, surrounding alveoli filled with mucoid secretion and desquamated cells   |   |
| Wood and Pierson, <sup>20</sup> 1945                        | 57  | F   | Right lung on section had multiple small grayish millary nodules Left lung similar<br>Microscopic Alveoli lined by columnar cells forming papillae, stroma intact   | "columnar epithelial cells appear to arise de novo from the alveolar walls"   |
| Ikeda, <sup>46a</sup> 1945                                  | 59  | F   | In each pleural cavity from 2,000 to 3,000 cc of clear fluid Right lung 500 Gm, left, 750 Gm, on section parenchyma was studded with whitish tumors<br>Microscopic Papillary growth arising from the alveolar lining  | "the tumor can originate from the lining cells of the alveoli and is epithelial in character"   |
| Geever, Carter, Neubuerger and Schmidt, <sup>44b</sup> 1946 | 17  | F   | Left lung and middle and lower lobes of right lung occupied by grayish tumor nodules varying from pinhead size to diameter of 10 cm<br>Microscopic Alveolar walls lined by columnar cells with rare mitotic figures   | "Although the majority of alveolar cell tumors have been definitely malignant, some were borderline or histologically benign"                           |
| Paul and Ritchie, <sup>21</sup> 1946                        | 68  | F   | Lower lobe of left lung studded with 2 mm nodules Bronchiectatic cavities present bilaterally   | "It appears that the abnormal epithelium of adenomatosis is derived, in some cases at least, from bronchial epithelium"                                 |
| Osserman and Neuhof <sup>10</sup>                           | 44  | F   | Lobectomy, lower lobe, left lung, on section lung tissue completely replaced by grayish tumor bronchi pushed toward pleural surface, no endo bronchial tumor seen<br>Microscopic Basement membrane of tumor consisted of alveolar walls, cells cylindric, mucus containing  |   |
| Alexander, C M, and Foo Chu Arch Path 43 92 1947            | 56  | F   | Adhesions in right pleural space and none in left Lungs together weighed 2100 Gm Lower lobe of right lung voluminous, yellowish pink, with gelatinous cut surface Solitary nodule in left lung<br>Microscopic Tall columnar cells alveoli, nonciliated, no mitotic figures droplets of mucus in cytoplasm Nearly every alveolus contained fibrinopurulent exudate | "We believe that in our case the tumor was most likely of multicentric origin since there was no demonstrable invasion of lymphatic vessels"            |
| Simon M A Am J Path 23 413, 1947                            | 70  | F   | Right thoracic cavity partially obliterated by fibrous adhesions less extensive adhesions on left Cut surfaces gray, consolidated Friedländer's bacilli cultured<br>Microscopic Majority of alveoli filled with polymorphonuclear leukocytes walls lined by single or pseudostratified layers of tall columnar epithelial cells                                   | "Pulmonary adenomatosis and alveolar cell tumors may be regarded as unusual forms of pulmonary carcinoma presumably arising from alveolar lining cells" |

TABLE 3—Cases of Cancerous Pulmonary Adenomatosis Collected from the Literature

| Author  | Age, Sex | Thoracic Findings   | Metastases  | Authors' Interpretation   |
|---|----------|---|---|---|
| Smith and Gault, <sup>46b</sup> 1942                      | 48 M     | Right visceral pleura studded with grayish nodules. Complete consolidation of right lung resembling hepatization.<br>Microscopic Alveolar walls show moderate fibrosis and capillary dilatation, lining cells large and polyhedral.   | Right visceral pleura, pulmonary lymphatic channels, peribronchial and peritracheal lymph nodes                                     | "Indication of possibility that cells are of actual alveolar lining cell origin with tendency toward adenocarcinoma"  |
| Dacie and Hoyle, <sup>11</sup> 1942                       | 54 M     | Pleural cavities partially obliterated bilaterally. Both lower lobes, right middle lobe, left upper lobe and lingula diffusely affected by changes suggesting chronic confluent bronchopneumonia.<br>Microscopic Epithelial proliferation within existing alveolar structure, alveolar walls intact, papillae numerous, in some areas, mucin secreting cells. | Invasion of pleura  | "they (tumors) appear to arise from alveoli of the lung rather than by extension from the bronchioles or bronchi"   |
| Wood, E. H., Jr. Radiology 40 193, 1943                   | 52 F     | Lungs appeared to be in state of diffuse gray hepatization, composed of lobular pinkish tan tumor nodules.<br>Microscopic Alveoli lined by simple or pseudostratified columnar tumor cells, few mitotic figures, numerous papillae, local invasion and destruction of alveolar septums.   | Local invasion and destruction of alveoli   | Inclined to feel there is some relation between lipoid pneumonia and carcinoma arising from epithelial lining cells of lung   |
| Herbut, <sup>45a b</sup> 1944                             | 50 F     | Bilateral adhesions. Entire right lung consolidated and gray, cut surface homogeneous gray, with nodules at periphery area of early necrosis in upper lobe. Left lung studded with circumscribed and confluent nodules.<br>Microscopic Alveoli lined with one or more layers of cuboidal or columnar cells and septums thickened.                             | Peribronchial and perivascular lymphatic channels, pericardium, mediastinal lymph nodes, liver, adrenal glands and vertebral marrow | "It is believed that the parent cell in all cases of primary carcinoma of the lung is the basal cell of the bronchial or bronchiolar mucosa. The distribution of the subsequent tumor is dependent upon the further differentiation of the cells. If they are cuboidal or columnar they will regularly line the septa producing the well known alveolar arrangement." |
|   | 48 F     | Adhesions in upper right pleural cavity. Entire right lung diffusely consolidated with grayish white tumor tissue. Left lung studded with pinhead size grayish nodules.<br>Microscopic Alveoli regularly lined by tall cuboidal cells.  | Mediastinal lymph nodes, pericardium and liver  |   |
|   | 52 F     | Adhesions in left pleural space. Entire left lung infiltrated with discrete and coalescing gray nodules. Right lung essentially similar.<br>Microscopic Tall columnar cells lined alveoli, considerable sloughing of cells in alveolar spaces.  | Tumor cells present in lymphatic vessels  |   |
| Ikedu, <sup>46a</sup> 1945                                | 52 F     | On section right lung consolidated with grayish white tissue from region of hilus. Left lung studded with nodules.<br>Microscopic Papillary growth arising from alveolar lining cells tall columnar.  | Both lungs regional lymph nodes, spleen, liver, adrenal glands, spine and pelvis  | "The path leading to the fully developed alveolar cell carcinoma of the lung may be assumed to begin as local hyperplasias, often in multiple centers, later developing into benign adenomatosis and finally becoming carcinomatous."   |
|   | 69 F     | (No description given)  | Regional lymph nodes and pleura   |   |
| Geever Carter, Neuberger and Schmidt, <sup>14b</sup> 1945 | 49 M     | Both lungs studded with firm gray nodules, occasionally coalescent.<br>Microscopic Nodules composed of anaplastic cells lining alveolar walls and forming papillae.   | Liver, adrenal glands, hilar lymph nodes  | "Although the majority of alveolar cell tumors have been definitely malignant, some were borderline or histologically benign"   |
|   | 53 F     | Both lungs studded with gray nodules which occasionally encroached on pleura.<br>Microscopic Alveolar walls thickened and lined by cuboidal cells.  | Brain pulmonary lymphatic vessels   |   |
|   | 76 M     | Left lung occupied by grayish yellow and white tumor nodules.<br>Microscopic Alveoli lined by highly anaplastic cells with many mitotic figures, scattered areas of necrosis.   | Regional and peripancreatic lymph nodes, pancreas, adrenal glands and kidneys masses of tumor cells in lymphatic channels           |   |
|   | 58 M     | Left pleura studded with nodules. On section upper lobe of left lung solid, pale gray, lower lobe contained a few nodules. No tumor in right lung.<br>Microscopic Alveolar walls lined by cuboidal or cylindrical cells with occasional mitotic figures and giant cells.  | Implants on pleura and pericardium, lymphatic channels contained tumor cells  |   |

TABLE 3—Cases of Cancerous Pulmonary Adenomatosis Collected from the Literature—Continued

| Author  | Age,<br>Sex | Thoracic Findings  | Metastases           | Authors' Interpretation   |
|---|-------------|--|----------------------|---|
| Fishman, A P, Epstein, B S, and Grayzel, D M<br>Am Heart J 30:309, 1945 | 20<br>F     | Right lung firm and rubbery throughout nodules in left lung, on section it resembled pneumonic consolidation, no necrosis<br>Left lung revealed a few circumscribed nodules<br>Microscopic Alveoli filled with tumor cells, stroma of tumor made up of alveolar walls, rare mitotic figures, marked endarteritis                               | Nodules in left lung |   |
| Wenger, <sup>46c</sup> 1945   | 60<br>F     | Adhesions present bilaterally On section right lung firm, grayish yellow, with appearance of hepatization Left lung studded with nodules, confluent in upper lobe<br>Microscopic Alveoli lined by epithelial cells, with occasional formation of papillae  | Liver                | Tumor appeared to be multicentric and most likely originated in cells lining pulmonary alveoli  |
| Simon, M A<br>J Clin Path 17:783, 1947                                  | 39<br>M     | Right pleural cavity completely obliterated by dense adhesions Cut surface of right lung composed of pinkish gray tumor tissue Left lung also involved<br>Microscopic Alveoli lined by single and sometimes pseudostratified layers of tall columnar cells, papillae common, mitotic figures rare Metastases were identical with tumor in lung | Bran                 | "Although controversial the evidence suggests that this tumor is multicentric in origin and is probably derived from lining epithelial cells" |

reversed sex incidence of 1:125 in this series of cases of adenomatosis resembles that of bronchial adenoma more closely than that of bronchogenic carcinoma<sup>19</sup>

It appears, moreover, that the statement that the majority of alveolar cell tumors are definitely cancers<sup>14b</sup> is borne out by these data. Neuburger and Geever<sup>1</sup> estimated that 25 per cent metastasize to the regional lymph nodes and another 23 per cent to distant sites. It is possible that thorough histologic examination of the peribronchial and hilar nodes would reveal that the suggested incidence of metastases is too conservative.

#### THE CHARACTERISTIC LESION

The following gross and microscopic descriptions are a composite of details drawn from the literature and from the material studied at the Army Institute of Pathology.

*Gross Description*—When death has resulted from pulmonary adenomatosis the pleural space is usually partially or completely obliterated by fibrous adhesions. The pleural cavity sometimes contains fluid, which is usually clear and may total as much as 3,300 cc (case 9). The lungs may vary considerably in weight, recorded weights ranging from 356 Gm<sup>20</sup> to 2,750 Gm<sup>5</sup>. The lungs are voluminous and tend to maintain their contours when the chest is opened. The visceral and parietal pleurae may be studded with gray to grayish pink nodules.

<sup>19</sup> Clerf, L. H., and Bucher, C. J. Ann Otol Rhin & Laryng 51:836, 1942.  
Holt, S. W. Mil Surgeon 99:528, 1946.

<sup>20</sup> Wood, D. A., and Pierson, P. H. Am Rev Tuberc 51:205, 1945.

It has become a common practice to describe the gross distribution of these tumors as nodular (miliary), diffuse or a combination of these two forms. Distribution may vary from involvement of a single lobe of one lung to all lobes of both lungs.

In the nodular variety, the cut surface is studded with tumor foci, ranging from minute to coalescing nodules, which may occupy an entire lobe. Peripherally the larger areas are irregular, and cut surfaces are yellowish white to grayish pink. The consistency is soft and often distinctly mucoid.

The diffuse form is characterized by homogeneous involvement of extensive areas of the parenchyma, but a suggestion of nodulation often is noted at the periphery of the neoplasm. Areas of necrosis of the tumor are exceedingly rare, but changes due to superimposed infection may simulate necrosis of tissue.

TABLE 4—*Doubtful Cases of Pulmonary Adenomatosis Collected from the Literature*

| Author                                       | Age, Sex | Thoracic Findings  | Metastases   | Author's Interpretation         |
|--|----------|--|--|---------------------------------|
| Sanda, E. Casop<br>lák, česk 80<br>237, 1941 | 65<br>F  | Pulmonary tissue firm and on section resembled pneumonic hepatization<br>Microscopic "Cancerous pneumonia"   |  | Alveolar carcinoma              |
|  | 43<br>M  | (No description given)   | Mediastinum  | Alveolar carcinoma              |
| Ikeda, <sup>46a</sup> 1945                   | 56<br>M  | Numerous flat nodules on pleura of right lung. On section, surface of right lung studded with grayish white nodules. Left lung essentially the same. | Secondary bronchus, (obstructed by growth), brain and meninges | Alveolar cell carcinoma of lung |

Intervening lung tissue is often hyperemic and shows evidence of inflammation. On compressing the lung, mucinous material exudes, sometimes associated with pus, for multiple abscesses and bronchiectasis occasionally complicate the picture. The gross appearance may be easily confused with that of the chronic granulomas of the lung which occur in tuberculosis, coccidioidomycosis and histoplasmosis, or with that of secondary neoplasms, especially those arising in the gastrointestinal or the genitourinary tract, or with that of such conditions as Boeck's sarcoid, leukemic infiltration or parasitism. The gross specimens of lungs available for study in this group often suggested the gray hepatization of lobar pneumonia. On the other hand, on gross examination a case of extensive areas of unresolved pneumonia was misinterpreted as one of adenomatosis.

*Microscopic Description*—The histologic picture is essentially the same in both the nodular and the diffuse form. There are variations in the pattern, ranging from simple investment of alveoli to complicated

arrangements resulting from extensive intra-alveolar proliferation with rupture of alveolar walls and coalescence of spaces

In the simplest form the alveolar walls are lined by a single layer of cuboidal or cylindric cells, which are usually nonciliated<sup>21</sup> The cells are remarkable for their monotonous regularity of structure and arrangement The cytoplasm is faintly eosinophilic, and special stains often reveal mucin-like material both in the cells and in the alveolar spaces The nuclei are usually situated at the bases of the cells, are oval and have a distinct nuclear membrane The chromatin has a powdery appearance, and there is a single large nucleolus, which stains purple to cherry red with Masson's stain Mitotic figures are exceedingly rare

In older tumors the behavior of the cells is different Their free surfaces become irregular, and it is common to see nuclei at the tips of the cells instead of at the bases Indeed, irregularity may become so prominent that some have described "giant-cell-like masses"<sup>22</sup> In this phase the cells show definite proliferative tendencies, with formation of papillary processes and rare mitotic figures The papillary proliferation may become so profuse that the limiting alveolar walls are ruptured, with coalescence of the papillary neoplastic tissue The alveolar spaces contain free cells, which appear to be derived from the investing epithelium, however, because they are unaffected by restraining influences they are rounded In some cases the spaces are almost filled with material which stains red with mucicarmine If secondary infection occurs, as it frequently does, polymorphonuclear leukocytes become numerous

The peribronchial, perivascular and pleural lymphatic channels may be lined by cells identical to those investing the alveoli, and occasionally lymphatic emboli of tumor cells are identified At times it is possible to demonstrate a gradient from early investment of alveoli to areas of frank cancer<sup>11</sup>

In the early phases the stroma of the tumor consists of normal alveolar walls In the later lesions, especially where inflammation has occurred, there is considerable fibrosis Squamous metaplasia is only rarely associated with fibrosis of alveolar walls and septums Extensive endarteritic changes of the pulmonary arteries may be seen in older lesions By the time autopsy is performed, the neoplastic proliferation has usually progressed to involve the peribronchial tissues Careful examination will reveal that the respiratory epithelium is intact, although there may be compression of the bronchial tree by the surrounding neoplastic mass

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21 Paul, L. W., and Ritchie, G. *Radiology* 47:334, 1946

22 Musser, J. H. *Univ. Pennsylvania M. Bull.* 16:289, 1903



Metastases may be present in pulmonary lymphatic channels and hilar lymph nodes and at distant sites. Their histologic appearance simulates that of adenocarcinoma, however, necrosis is almost always lacking, and the cells closely resemble those investing the alveoli.

#### ETIOLOGIC CONSIDERATIONS

The lungs of sheep affected with jagziekte<sup>23</sup> have a remarkable similarity to human lungs seen in this series (fig 14). The same observation has been made by others.<sup>24</sup> Jagziekte (from *jagt*, to drive, and *Ziekte*, a sickness) has also been called verminous pneumonia,<sup>25</sup> epizootic adenomatosis<sup>26a</sup> and Montana progressive pneumonia.<sup>27</sup> Geographically, it has been reported in South Africa,<sup>28</sup> France,<sup>29</sup> Great Britain,<sup>30</sup> Iceland and Germany,<sup>26b</sup> the United States,<sup>31</sup> and Peru.<sup>32</sup> In 1927 Cowdry and Marsh<sup>27</sup> conducted an inquiry to determine whether the disease occurred in Argentina, Australia and New South Wales, but no cases resembling those of jagziekte had been reported in those sheep-raising countries.

Examination of a pulmonary lesion of a goat<sup>33</sup> revealed changes characteristic of jagziekte (fig 15), however, nothing referring to caprine pulmonary adenomatosis has been found in the literature, and Geever has stated that in his experience the condition is unique.<sup>34</sup>

Numerous theories as to the cause of jagziekte have been proposed but have been either rejected or not substantiated. Protozoa resembling the crescent of *Plasmodium falciparum* were described by Robertson.<sup>35</sup>

23 Case from Registry of Veterinary Pathology, Army Institute of Pathology Accession 185361, contributed by C. L. Davis, D.V.M.

24 (a) Sims, J. L. Arch. Int. Med. **71** 403, 1943. (b) Neuburger.<sup>4</sup> (c) Bonne.<sup>12</sup> (d) Wood and Pierson.<sup>20</sup>

25 M'Fadyean, J. (a) J. Comp. Path. & Therap. **7** 31, 1894. (b) **33** 1, 1920.

26 Dungal, N. (a) Proc. Roy. Soc. Med. **31** 497, 1938. (b) Am. J. Path. **22** 737, 1946.

27 Cowdry, E. V., and Marsh, H. J. Exper. Med. **45** 571, 1927.

28 (a) Mitchell, D. T., in Third and Fourth Annual Reports of the Director of Veterinary Services, Union of South Africa 1915, 585-614. (b) Cowdry, E. V. J. Exper. Med. **42** 335, 1925. (c) De Kock, G., in Fifteenth Annual Report of the Director of Veterinary Services, Union of South Africa, October 1929, p. 611 and (d) p. 1169.

29 Aynaud, M., Peyron, and Falchetti. Compt. rend. Acad. d. sc. **195** 342, 1932.

30 (a) Taylor, E. L. Proc. Roy. Soc. Med. **31** 505, 1938. (b) M'Fadyean.<sup>25</sup>

31 (a) Creech, G. T., and Gochenour, W. S. J. Agric. Research **52** 667, 1936. (b) Cowdry.<sup>28b</sup> (c) Cowdry and Marsh.<sup>27</sup>

32 Caparo, A. C. Bol. escuela van de cienc. vet. **1** 27, 1945.

33 Case from Registry of Veterinary Pathology, Army Institute of Pathology Accession 185359, contributed by C. L. Davis, D.V.M.

34 Geever, E. F. Personal communication to the author.

35 Robertson, W. J. Comp. Path. & Therap. **17** 221, 1904.

Cowdry<sup>28b</sup> was unable to demonstrate spirochetes. M'Fadyean<sup>25a</sup> suggested that in Great Britain verminous pneumonia was caused by a nematode, *Strongylus rufescens*, however, the common lung worm, especially in Iceland,<sup>26b</sup> is *Muellerius capillaris*. It is the consensus that these nematodes are not the etiologic agent, but they may be a predisposing factor<sup>28b</sup>.

Pulmonary growths resembling jagziekte have been encountered in numerous species of animals as well as in man. Grumbach<sup>36</sup> described alveolar epithelial hyperplasia in guinea pigs following injection of diphtheroid organisms. Cowdry<sup>27</sup> had the opportunity of comparing Grumbach's slides with those from cases of jagziekte and was able to verify their close similarity. Jagziekte of horses and mules was described by Theiler<sup>37</sup>. Experimental contact, injections of emulsified organs and pericardial fluid, and drenching in urine did not convey the disease to healthy horses and mules. He conclusively proved that the equine disease was caused by a plant, *Crotalaria dura*. Although his microscopic descriptions and photographs do not entirely convince one that the changes are identical with those seen in sheep affected with jagziekte, his work is remarkable in that he has presented adequate evidence that a noxious plant as well as specific micro-organisms can produce a typical pulmonary disease. De Kock,<sup>28c, d</sup> in experiments conducted at Onderstepoort and Tweespruit, South Africa, was unable to reproduce jagziekte in sheep with contact. He states that it can safely be said that jagziekte does not belong to the category of ordinary infectious diseases.

Olafson and Monlux<sup>38</sup> examined a cat infected with *Toxoplasma*, and found the alveoli of the lung lined by large epithelial cells, which gave the affected portions an adenomatous appearance. The alveolar walls were thickened and hyperemic.

Tyzzar<sup>39</sup> and others<sup>40</sup> described "papillary cystadenoma" occurring in the lungs of mice. The observation has since been made that such tumors are exceptionally common in this species, although uncommon in other mammals with the exception of sheep.

It has been shown experimentally that carcinogenic hydrocarbons can produce pulmonary tumors in mice. The pulmonary lesions of mice described recently by Lorenz and Stewart<sup>41</sup> closely resemble the human

36 Grumbach, A. Bull Assoc franç p l'étude du cancer **15** 213, 1926

37 Theiler, A., in Seventh and Eighth Reports of the Director of Veterinary Research Department of Agriculture, Union of South Africa, 1918, p. 59

38 Olafson, P., and Monlux, W. S. Cornell Vet **32** 176, 1942

39 Tyzzar, E. E. J. M. Research **21** 479, 1909

40 (a) Slye, M., Holmes, H. F., and Wells, H. G. J. M. Research **25** 417, 1914 (b) Wells, H. G., Slye, M., and Holmes, H. F. Cancer Research **1** 259, 1941 (c) Grady, H. G., and Stewart, H. L. Am. J. Path. **16** 417, 1940

41 Lorenz, G., and Stewart, H. L. J. Nat. Cancer Inst. **7** 227, 1947

type of adenomatosis. On the other hand, the tumors reported by Grady and Stewart<sup>40c</sup> were actually papillary adenomas and distinct from pulmonary adenomatosis of man.

Dungal,<sup>26b</sup> whose experimental work was carried on in Iceland where jagziekte is a serious problem, found that the infection was readily brought about when healthy lambs were exposed to the exhaled breath of sick sheep. He was unsuccessful in reproducing jagziekte by introducing bronchial secretions or ground tissues from affected sheep into the lungs of healthy animals. However, if sheep were already infected with lung worms it was possible to produce the lesions of jagziekte with extracts of diseased tissue, if this was given in conjunction with intratracheal injections of cultures of bacteria that cause pneumonia in sheep. In spite of the prevalence of jagziekte in the island, Dungal stated that he had never seen a similar lesion in the human lung in the numerous autopsies which he had performed. This observation is in disagreement with those of other investigators, who have described lesions of the human lung closely resembling those of jagziekte.

Experimental transmission was attempted with material from 2 human subjects,<sup>42</sup> but the results obtained with that of one are alone available.<sup>24a</sup> The pulmonary tissue of this one injected into rabbits, guinea pigs and monkeys had no effect. It should be noted that sheep were not included, and the species used, with the possible exception of guinea pigs, probably have low tissue susceptibility to this condition.

To date there is nothing in the literature to indicate that human beings contract jagziekte from animals, and an infectious origin cannot be determined from the evidence available.<sup>43</sup>

#### HISTOGENESIS

The controversy regarding the genesis of this tumor traverses the gamut from claims for a particular cell of origin<sup>44</sup> to the denial of the very existence of such a tumor entity.<sup>45</sup> The recent literature, however, has in the main shown a significant trend toward acceptance of the theory of an alveolar epithelial origin.<sup>46</sup>

If one is to assume that the tumor arises in the peripheral portion of the lung, it would be logical to consider the evidence for the presence

42 Wood and Pierson<sup>20</sup> Sims<sup>24a</sup>

43 Dungal<sup>26b</sup> Creech and Gochenour<sup>31</sup> Theiler<sup>37</sup>

44 Neubuerger and Geever<sup>1</sup> Neubuerger<sup>4</sup>

45 Herbut, P. A. (a) *Am J Path* **20** 911, 1944, (b) *Arch Path* **41** 175, 1946 (c) Arkin and Wagner<sup>17</sup>

46 (a) Ikeda, K. *Am J Clin Path* **15** 50, 1945 (b) Smith, L. W., and Gault, E. S. *Essentials of Pathology*, ed 2, New York, D Appleton-Century Company, Inc., 1942 (c) Wenger, F. *Prensa méd argent* **32** 44, 1945 (d) Taft, E. B., and Nickerson, D. A. *Am J Path* **20** 395, 1944 (e) Bell<sup>13</sup>

or the absence of an alveolar lining. The work of investigators has yielded diametrically opposed results, as evidenced in the comprehensive reviews of Miller<sup>47</sup> and Loosli<sup>48</sup>. Miller recognized that the greatest obstacle to an understanding of alveolar epithelium was the impossibility of dissecting it off. However, in certain pathologic processes, for example, the outpouring of a serous exudate behind the epithelium, as in pneumonia, atelectasis<sup>49</sup> and pulmonary edema of mitral stenosis, he observed that the fluid peeled the epithelium from the alveolar walls. He concluded that the lining of the alveolar walls was a continuous epithelium. This mechanism of *vis a tergo* had also been described in 1936 by Gazayerli,<sup>50</sup> who found that certain substances when injected into the pleural sac rendered the alveolar lining visible just as disease processes do. Cooper<sup>51</sup> studied the histologic evidence presented in human material and concluded that there is a fetal alveolar epithelium which persists but becomes attenuated when air distends the lungs. This same observation has been made by Bensley and Groff<sup>52</sup> and by Zeldes<sup>53</sup>.

Bremer<sup>54</sup> believed that the alveoli were lined with a continuous layer of epithelium of entodermal origin and that the cytoplasm of the component cells extended over the alveolar capillaries with flange-like processes.

Palmer and others<sup>54</sup> were of the opinion that there was a discontinuous lining epithelium of the alveolus, and Sprunt,<sup>54</sup> that there were two kinds of cells—one a phagocytic mesenchymal cell, the other an epithelial lining cell.

Loosli<sup>48</sup> and others<sup>55</sup> were unable to find irrefutable evidence of the existence of an epithelial lining. Macklin<sup>56</sup> observed that because a lining epithelium could not be demonstrated, the number of those denying its presence had increased. He recognized that if this opinion were generally adopted one would have to reevaluate the current concepts of the alveolus so as to regard it as "a functional interstitial emphysema." According to Cooper,<sup>51</sup> "these views are incompatible with histological, pathological and embryological evidence, and the arrangement of free spaces communicating with the exterior and in

47 Miller, W. S. *The Lung*, ed. 2, Springfield, Ill., Charles C. Thomas, Publisher, 1947.

48 Loosli, C. *Am J Anat* **62** 375, 1938.

49 Lohlein, M. *Verhandl. d. deutsch. path. Gesellsch.* **12** 111, 1908.

50 Gazayerli, M. E. *J. Path. & Bact.* **43** 357, 1936.

51 Cooper, E. R. A. *J. Path. & Bact.* **47** 105, 1938.

52 Bensley, S. H., and Groff, M. B. *Anat. Rec.* **64** 27, 1935.

53 Zeldes, M. *Arch. Path.* **20** 748, 1940.

54 Cited by Macklin<sup>56</sup>.

55 (a) Lang, F. J. *Virchows Arch. f. path. Anat.* **275** 104, 1930. (b) Norris, R. F., Kochenderfer, T. T., and Tyson, R. M. *Am J. Dis. Child* **61** 933, 1941.

56 Macklin, C. C. *J. Thoracic Surg.* **6** 82, 1936.

contact with thin-walled capillaries does not accord with histological findings in general"

Bloom<sup>54</sup> expressed the belief that mesenchymal cells may assume an epithelial arrangement and that the lining separated from the alveolar wall in pathologic conditions may be composed of mesodermal cells and exudate. Fried<sup>57</sup> regarded cells found in alveoli as mesodermal phagocytes originating in the alveolar septums. Lang<sup>55a</sup> introduced the term "septal cell" to indicate a special phagocyte belonging to the reticuloendothelial system, equivalent to the epicyte (Clara). Macklin<sup>56</sup> recognized the possibility that these cells may become the nucleus of a primary pulmonary carcinoma. Neuburger and Geever<sup>1</sup> proposed that the so-called alveolar cell tumor was derived from septal cells. It is well to note that Miller was unconvinced that this cell was anything more than an altered epithelial lining cell, or at most a mononuclear leukocyte, and Herbut<sup>45a</sup> was unable to find any convincing evidence that the septal cell was the parent cell of the tumor cell.

Dungal<sup>26b</sup> inclined to the view that in jagzłekte the mononuclear exudate cells were derived from the alveolar lining and that the lining cells could proliferate, without losing contact with the alveolar wall, to form an epithelial tuft which might be the beginning of an epithelial growth. De Kock<sup>28c</sup> concluded from the results of his studies of sheep that the majority of proliferations arose from alveolar epithelium.

Studies in mice have been numerous, and Furth and Furth,<sup>58</sup> Grady and Stewart<sup>40c</sup> and others<sup>59</sup> were of the opinion that the papillary tumors arose in the alveoli.

#### RELATION OF PULMONARY ADENOMATOSIS AND CARCINOMA

It is a misconception that regional lymph node or generalized metastases are never present with pulmonary adenomatosis. However, Richardson<sup>8</sup> was of the opinion that these tumors represented a distinct type of epithelial growth without formation of metastases. Ikeda<sup>46a</sup> considered only one of the tumors he reported a typical example of what he called alveolar cell carcinoma of the lung, for, among other criteria, there were no regional lymph node or distant metastases. Paul and Ritchie<sup>21</sup> discussed a "benign" and a "malignant" form of pulmonary adenomatosis.

The case reports of Malassez<sup>60</sup> and Musser<sup>22</sup> are commonly accepted as the first to present the nodular and the diffuse type, respectively, and in both regional lymph node metastases were described.

57 Fried, B. M. Arch Path **17** 76, 1934, cited by Macklin<sup>56</sup>

58 Furth, J., and Furth, O. B. Am J Cancer **34** 169, 1938

59 McDonald, S., Jr., and Woodhouse, D. L. J Path & Bact **54** 1, 1942  
Tyzzer<sup>39</sup> Slye and others<sup>40a</sup> Wells and others<sup>40b</sup>

60 Malassez, L. Arch de physiol norm et path **3** 353, 1876

It is the accepted procedure to base criteria for any condition on the original description. If time and experience demonstrate errors, then new or modified criteria are accepted. However, there has been no evidence presented in seventy-one years which alters in any way the original descriptions, therefore, they may be considered classic.

In 1907 Helly<sup>2</sup> discussed the possibility that the pulmonary lesion which he observed in a 43 year old woman may have been cancer. In 1930 Oberndorfer<sup>62</sup> stressed the fact that there is no distinct borderline between alveolar investment, which histologically appears benign, and frank carcinoma of the lung. He concluded that in the lung, as in the parenchymatous cells of the liver, sudden mutations of the epithelium may arise in several areas leading to "blastoma" formation.

Bonne,<sup>12</sup> in 1939, suggested the term carcinosis for the pulmonary lesion under discussion. He was puzzled by the histologically "benign" appearance of the tumor and the contradictory quality of "malignancy" represented by invasion of pleura and death of the patient. Dacie and Hoyle,<sup>11</sup> in reporting a case in 1942, illustrated "a fine gradation histologically from benign 'adenomatosis' to definite invasive carcinoma." The same year Smith and Gault<sup>40b</sup> included in their textbook an account of a case in which they described innumerable mitotic figures and stated, "some suggestion of lumen formation is seen indicating the possibility that the cells are of actual alveolar lining cell origin with a tendency toward adenocarcinomatous formation." In 1943 Bell<sup>18</sup> wrote "There is no good reason to doubt that hyperplasia of the alveolar epithelium may give rise to localized or diffuse growths which may form metastases." In 1944 Geever, Carter, Neubuerger and Schmidt<sup>14b</sup> commented that the majority of "alveolar-cell tumors are malignant." Finally, Paul and Ritchie<sup>21</sup> concluded from evidence in one of their cases that "adenomatosis" must be regarded as a pre-cancerous lesion.

*Comparative Pathology*—Aynaud, Peyron and Falchetti,<sup>29</sup> who examined numerous cases of verminous pneumonia originating in France, concluded that the process was a real tumor. Dungal had the opportunity of examining the sections of jagziekte studied by this group and stated "there were undoubted metastases in a lymph gland." Innes<sup>63</sup> was also convinced that jagziekte was neoplastic in nature, and De Kock,<sup>28c</sup> that serious consideration should be given its "malignant" potentialities. De Kock and others<sup>40c</sup> pointed out the similarity between jagziekte and spontaneous pulmonary tumors of mice. In 1911

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61 Footnote deleted by the author

62 Oberndorfer, S. Virchows Arch f path Anat **275** 728, 1930

63 Innes, J R M, in discussion on Taylor<sup>30a</sup>

Haaland<sup>64</sup> examined a large series of primary pulmonary tumors of mice and concluded that they were "malignant"

Numerous investigators have induced pulmonary tumors in mice with a variety of irritants<sup>65</sup> Campbell<sup>66</sup> observed primary pulmonary tumors in mice exposed to dusts and tars of various kinds and was of the opinion that they usually originated from alveolar cells as "non-malignant" tumors and then changed more or less rapidly into "malignant" types, depending on the degree of irritation

#### COMMENT

*General Considerations*—The lesions seen in the lungs of sheep, goats, horses and mules, guinea pigs, cats and man must be interpreted with considerable caution. Numerous investigators have discussed the striking similarity between jagziente and alveolar cell tumor of the human lung. I have had the opportunity to compare sections from certain spontaneous and induced pulmonary tumors in mice<sup>41</sup> with those of goat, sheep and man, and it is apparent that the pulmonary lesions closely resemble one another. Therefore, it is possible that an identical pulmonary reaction takes place in these widely divergent species, and a review of the causes of adenomatosis in these species may shed light on the cause of this condition in man.

*Etiologic Factors*—It seems established that widely assorted irritants acting on the lung must produce a rather stereotyped response regardless of species, i. e., investment of the alveolar walls. The stimulating agents may be exogenous and include chemical fumes or other respiratory irritants, or they may be endogenous and include the extrapulmonary introduced carcinogens, ingestion of certain poisonous weeds, bacteria, protozoa and possibly viruses. There is evidence that investment of alveoli occurs in the human lung in a wide assortment of chronic pulmonary conditions, such as pneumonia, tuberculosis, atelectasis, psittacosis and chronic passive congestion. No etiologic factor has been proved to be specific. If a virus or viruses should be isolated, they would probably be either contributory or another one of the innumerable irritants. The possibility does exist that diverse irritants may prepare the "ground" for the action of a virus, but from the evidence available this seems unlikely.

*Histogenesis*—The origin of the epithelium-like tumor cells in pulmonary adenomatosis is still undetermined. However, the presumptive

64 Haaland, M. Fourth Scientific Report of the Imperial Cancer Research Fund. London, Taylor and Francis, 1911, Proc. Roy. Soc. Med. 83: 520, 1911.

65 Andervont, H. B., and Shimkin, M. B. J. Nat. Cancer Inst., 1: 225, 1940. Leiter, J., Shimkin, M. B., and Shear, M. J. ibid. 3: 155, 1942. Grumbach<sup>30</sup> Grady and Stewart<sup>10c</sup> Furth and Furth<sup>58</sup>

66 Campbell, J. A. Brit. J. Exper. Path. 18: 215, 1937.

evidence obtained from clinical, experimental and pathologic data leads me to conclude that a continuous alveolar lining probably exists

In no instance could the available evidence be interpreted as indicating that the alveolar investing cells arose from bronchi by a process of extension, as Herbut claimed. It is noteworthy that in 1 case the investing cells were found to be ciliated. This is an unusual finding, reported once before<sup>21</sup>. There is no reason to believe that these cells arose in the bronchi, ciliation is merely an expression of the pluripotential character of the parent cell. It has also been shown that the investing cells may be predominantly mucus-secreting and may look and act like goblet cells.

It is obvious that ciliated or goblet cells are not autochthonous to the pulmonary alveolar walls. As long as proof is lacking that they have their origin from the bronchial epithelium, it is my opinion that the alveoli contain some cells capable of attaining autonomy.

*Pathogenesis*—Comparison of material from human and experimental sources leads to the deduction that the cells which line the alveoli in many pathologic conditions have the same origin as the cells which are present in the so-called alveolar cell tumor. It is common knowledge that if the cause of pulmonary irritation is withdrawn the inflammation may resolve completely. If the irritation continues, the first changes are seen in the respiratory epithelium and the bronchial glands. Within the glands there is an increase in the number of goblet cells and apparent hyperplasia of the mucous glands, and a considerable quantity of glairy mucus exudes over the respiratory epithelium and acts as a protective coating. This is manifested clinically by increasingly abundant mucoid sputum.

Up to this point there is little if any disagreement among investigators. The following conclusions have been evolved by study of the monotonous repetition of the histologic picture in all chronic pneumonitides. If the irritation continues, a remarkable change takes place in the alveoli themselves, characterized by the appearance of alveolar investing cells. The investing lining cells are, in all probability, a recapitulation of the protective function displayed in the bronchi, already described.

It becomes apparent at this phase that the hyperplasia of the alveolar lining cells has reached a stage of "new growth," where it ceases to serve a useful function for the organism. It has been demonstrated that continued proliferation of the lining cells invests extensive areas of lung parenchyma and obviously acts as a barrier to normal physiologic function of the lungs. Thus the patient may literally "drown" in tumor tissue before the true cancerous propensity of the proliferation is made obvious by distant metastases, as in case 9.



## SUMMARY

"Pulmonary adenomatosis" is suggested as the preferable designation for so-called alveolar cell tumor of the human lung. If metastases occur, the term should be modified by adding the adjective "cancerous" (cancerous pulmonary adenomatosis).

Criteria proposed for the diagnosis of pulmonary adenomatosis are (1) alveolar cellular proliferation characterized by the appearance of tall columnar mucus-producing cells, (2) absence of an intrinsic tumor of the bronchial tree, and (3) absence of primary adenocarcinoma of any other part of the body.

No etiologic factor of pulmonary adenomatosis of man has been proved to be specific, the disease apparently is not infectious.

The majority of observers are of the opinion that pulmonary adenomatosis is an extrabronchial neoplasm with cancerous potentialities. In none of the cases presented was there conclusive evidence as to the exact site of origin.

Although these tumors appear histologically noncancerous, clinically they must be considered to be cancers, since they may kill by local growth or by metastasis.

## THE MEGAKARYOCYTE

### III Pseudothrombocytes

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CHICAGO

AS MAY be seen from Hittmair's<sup>1</sup> extensive review, there is no constituent of the hemopoietic tissue which was not at one time or another considered to produce thrombocytes. The interpretation of these structures varied from a complete cell to a mere precipitate. The morphologic features and distinctive reactions of the thrombocytes point to unity of function, form and origin. At present the megakaryocyte is almost unanimously accepted as their only source. Nevertheless the unity of their formative process is not generally recognized. Whereas the original theory of Wright assumes that the thrombocytes are liberated by disintegration of the mature, granulated megakaryocyte, other workers<sup>2</sup> have ascribed thrombocytopoietic activity also to the early, nongranulated stages. At this early phase pseudopodia are said to protrude, which when separated from the cell body either represent basophilic juvenile thrombocytes<sup>2c</sup> or produce a chromomere from nuclear chromatin particles which have been dislocated into them<sup>2b, d</sup>. These assumptions imply two significantly different processes: (1) the liberating of thrombocytes from the mature megakaryocyte, after completion of granulopoiesis, and (2) the segregating of the hyalomere from the basophilic cell, before the start of granulopoiesis, followed by secondary elaboration of the chromomere. Although Willi<sup>2b</sup> and Rohr and Koller<sup>2d</sup> assumed that the nuclear chromatin participates in both mechanisms, the succession of the formative steps remains inversed. The secondary production of granules in the pseudopodia, furthermore, is not in accordance with the granulopoiesis that occurs in the megakaryocyte described by me.<sup>3</sup> Granulopoiesis must be considered as a monocentric process, starting in the "functional area," which gradually expands over the cell body without any intervention of

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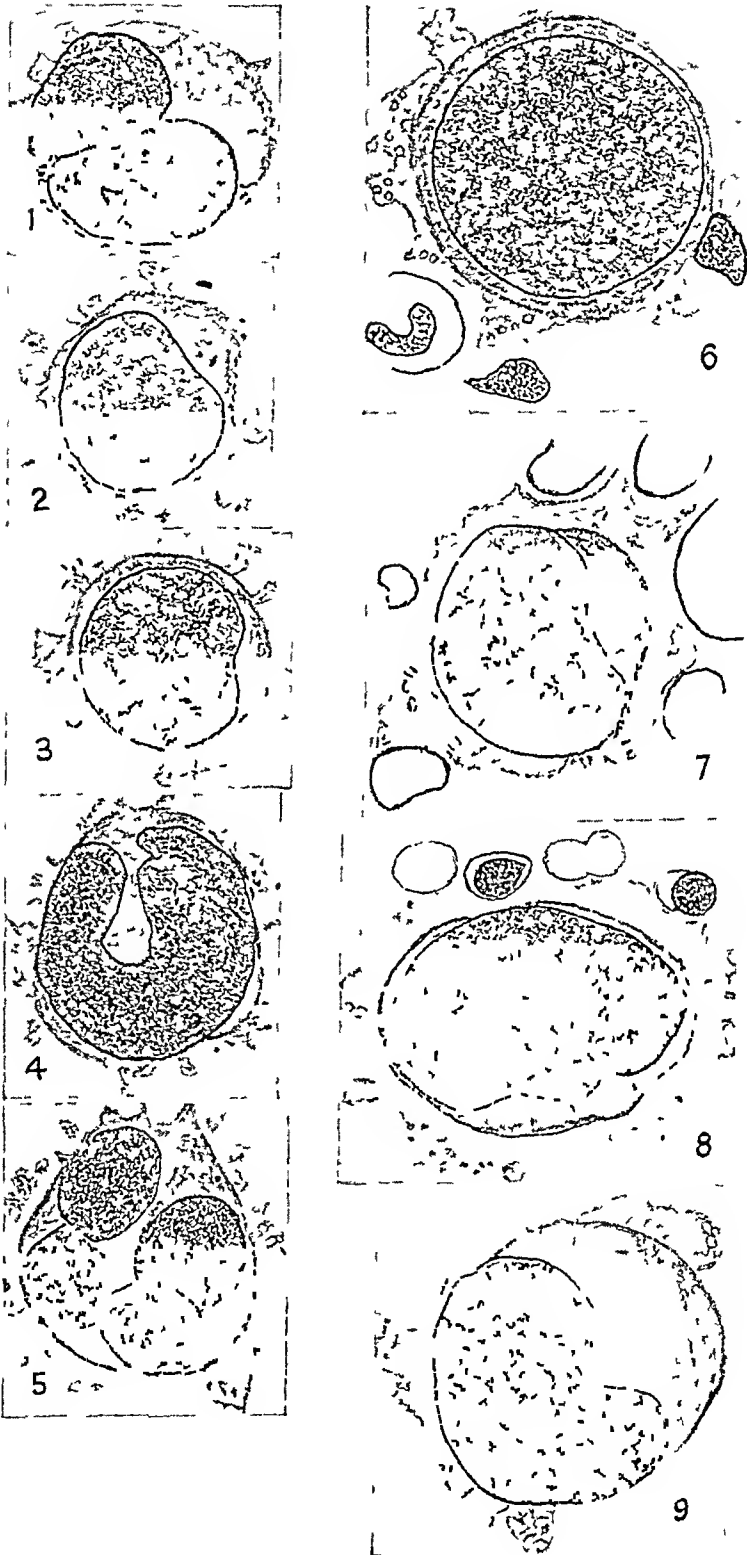
From the Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital

The Department is supported in part by the Michael Reese Research Foundation and the Hematology Research Foundation

1 Hittmair, A. *Folia haemat* **35** 156, 1927

2 (a) Medlar, E. M. *Folia haemat* **53** 397, 1935 (b) Willi, H. *ibid* **53** 426, 1935 (c) Juergens, R., and Graupner, H. *ibid* **57** 263, 1937 (d) Rohr, K., and Koller, F. *Klin Wchnschr* **15** 49, 1936 (e) DuBois, A. H. *ibid* **16** 22, 1937 (f) Downey, N., and Nordland, U. *Folia haemat* **62** 1, 1939

3 Schwarz, E. (a) *Arch Path* **45** 333 and (b) 342, 1948



Figures 1-9

(See legends on opposite page)

accessory granulopoietic foci or chromatin particles. For these reasons the aforementioned findings should permit another interpretation, which necessarily will reflect also on the pathologic changes of the thrombocytes. The following observations may contribute to the clarification of these controversial issues.

#### OBSERVATIONS

The studies were made on smears and sections of human marrow, which was obtained by sternal puncture from persons with either normal or morbid hemopoiesis. The usual staining methods were applied.

*Distribution of "Pseudopodia"*—Threadlike, fringed or lobiform excrescences are a common deformity of early megakaryocytes. Such "pseudopodia" appear on almost all the megakaryoblasts, are less readily observed on the promegakaryocytes and are rarely seen on the mature cells. The frequency or intensity of the fimbriation has no relation to the general condition of hemopoiesis or the number of thrombocytes. Numerous instances of fimbriation were observed also in thrombopenic purpura.

*Morphologic Aspects*—1 The number of excrescences varies from a single one to a wreath or corona of them (figs 1 to 5). Some may appear as a filiform fringe (fig 4), or may display clublike ends (figs 2 and 3), or may show quite irregular shapes, but every one of them has a narrow insertion on the surface of the cell. Other protrusions with a broader base are more or less lobiform (figs 1, 3, 6 and 7). Instead of pseudopodia, apposition-like formations frequently stretch along the cell outline, either replacing the lobes or being in continuity with them (figs 3, 6 and 9). These structures show an indefinite outline, are usually interrupted, and rarely and only in very immature cells do they form a continuous envelope around the cell (figs 7 and 8).

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Fig 1—Megakaryoblast with few excrescences

Fig 2—Megakaryoblast with many pseudopodia-like excrescences, one of them detached and simulating a basophilic thrombocyte

Fig 3—Megakaryoblast with many excrescences and some continuous appositions

Fig 4—Early promegakaryocyte with a corona of excrescences

Fig 5—Promegakaryocyte with many excrescences, some of them detached, the latter representing pseudothrombocytes

Fig 6—Promegakaryocyte with a nearly continuous envelope. Note the very large single nucleus resulting from a monocentric mitosis

Fig 7 and 8—Early megakaryoblasts, each with a continuous envelope and a small functional area

Fig 9—Maturing promegakaryocyte with a large functional area and a few lobiform excrescences

Note the sharp line of demarcation between the cell body and the pseudopodia in all figures. Magnification about 1,000

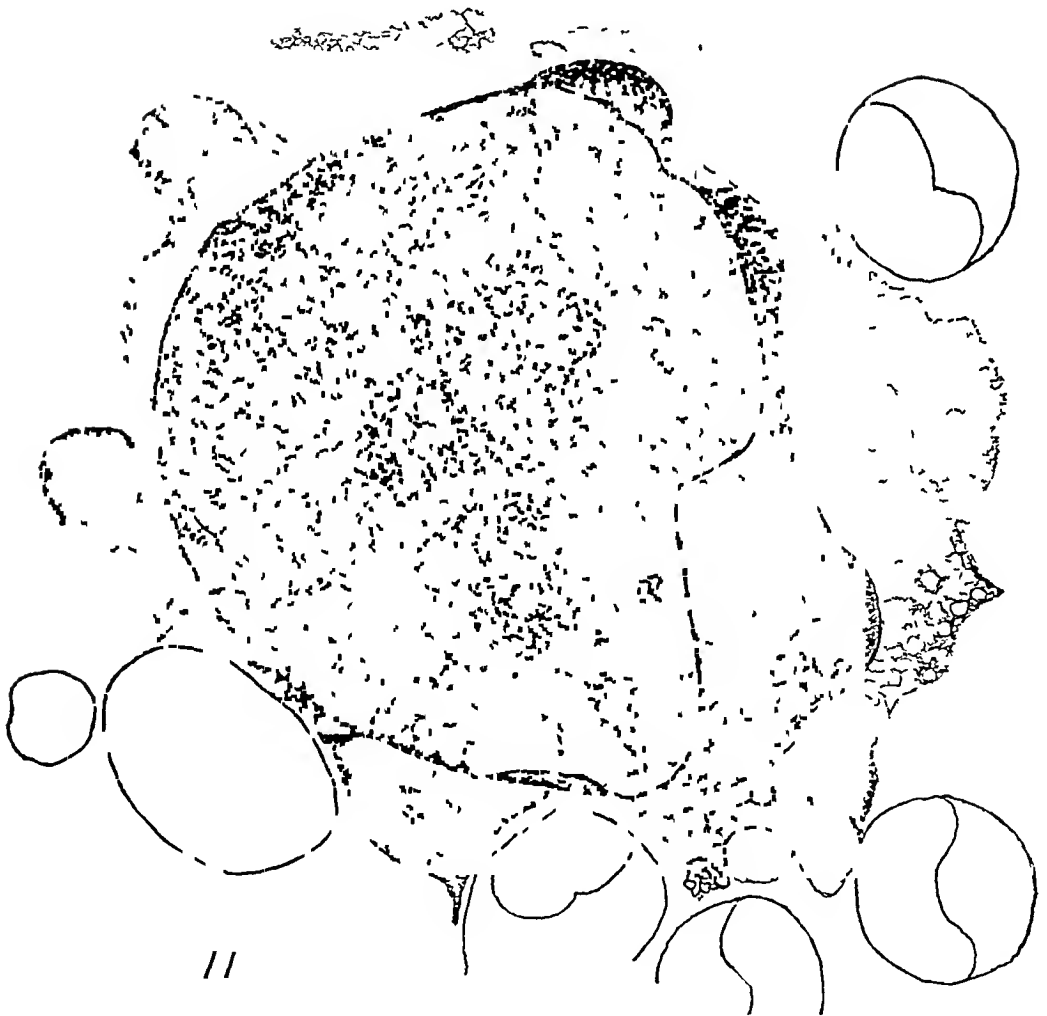
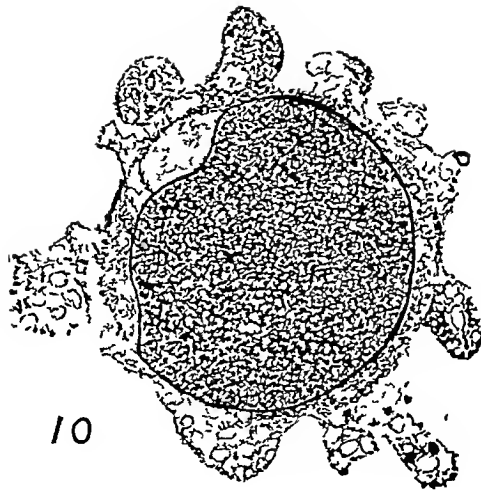
As far as they contain solid material the protrusions stain in various shades of blue, but always they are lighter than the cytoplasm. A sharp line of demarcation separates the bases of the protrusions from the cell body. In places where the denuded nucleus occupies a part of the cell contour, close observation reveals a thin blue line separating the bases of the protrusions from the nuclear membrane (figs 1, 4 and 5).

2 One type of excrescence shows basophilic threads and granules embedded in an almost colorless ground substance. The filaments form a loose and irregular meshwork and are composed of minute dotlike or beadlike granules, which at the point of intersection are often coarser and more conspicuously stained. In another type the more homogeneous ground substance stains distinctly blue and is perforated by numerous small holes varying from pinpoint size upward, producing a foamy appearance (figs 3 and 6). The holes, usually circular, contain no material visible either by stain or by refraction of light. The surface of the slide provides the background of these absolutely empty spaces. In order to show the structural differences, which are rather difficult to reproduce in drawings, figures 10 and 11 were drawn on the double scale. Not only the structural properties but the sharp line of demarcation and the well defined "functional area" are worth noticing.

In another configuration the foamy structure occupies either larger lobes<sup>4</sup> or a zone of varying breadth of the cytoplasm. In both cases the line of demarcation is absent. In figure 12, representing a promegakaryocyte in pluripolar mitosis, the dark foamy zone surrounds the central homogeneous and pale chromosomal field.

3 In many fields of the films, in places where the cells have not spread too far from each other the dried sap between the marrow cells shows small holes just the same as those described. If the cells are widely separated, the intercellular material is almost invisible. The holes are absent where the cells are tightly crowded. Variations in the thickness of the film and in the blood plasma dilution of the sap account for these differences. In the preparation of the smear the slides do not move either in the same plane or with constant velocity, and therefore pressure and suction alternate and drive air into the sap. These air bubbles are not retained in the thinner fluid layers but remain enclosed in the thicker and more viscous ones, thus forming a true foam. In the drying process the bubbles explode, leaving the round perforating holes. Moreover, the various physical conditions cause irregularities of the surface tension, a factor which probably is responsible for the filaments and the granular precipitations in the drying sap.

Applying this interpretation to the identical foamy appearance of many protrusions and parts of the cytoplasm of the megakaryocytes,



Figs 10 and 11—Megakaryoblasts with initial functional area. Note the many lobes, pseudopodia and some continuous appositions. The foamy structure and the continuous transitions from one type of excrescence to another are very clear. Note the distinct line of demarcation. The cells are drawn on a double scale in order to show the structural details. Magnification, about 1,000.

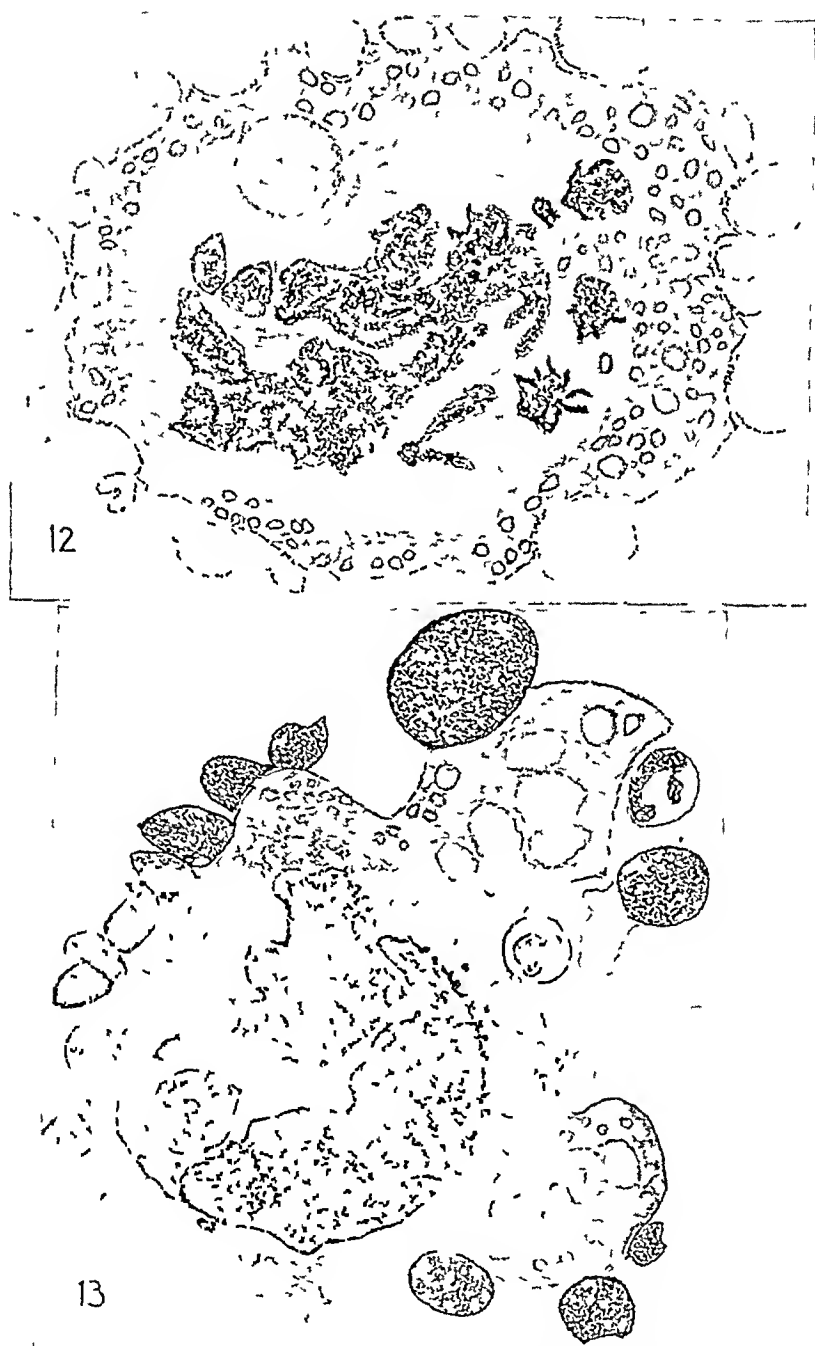


Fig 12—Megakaryoblast with a smashed pluripolar mitotic figure. The peripheral zone of the cell shows foamy structure. There is here no line of demarcation. The circular functional area is distinctly outlined. Magnification, about 1,000.

Fig 13—Mutilated mature megakaryocyte. Note the large lobes with holes and foamy structure. Here there is no line of demarcation. Magnification, about 1,000.

one perceives that these features do not represent a genuine structure or vacuolation. They are the result of pressure and stretching and of the viscosity of the material on which these forces act.

Obviously, the peripheral parts of the cell are foremost in yielding to these forces, because of the decrease of limiting obstacles toward the exterior. From these points of view the features of the cell drawn in figure 12 are easily understood, as well as the origin of the structures described as pseudopodia and lobiform protrusions. Furthermore, the presence or the absence of the demarcation line decides whether the lobes belong to a substance outside the cell surface or to the cell body itself, a distinction which will prove itself of principal significance.

The movements of the slides develop not only pressing and stretching but also shearing forces which lacerate the material and tear off more or less of it, thus forming the pseudopodia-like excrescences and the thrombocyte-like isolated corpuscles. Lobes with a broader base result in places where the forces are weaker. The appositions described are but the residues of the flattened material spared from the shearing off and from the laceration. Their lack of the foamy appearance suggests a lesser viscosity and consequently a stretching into thinner layers. That there are local variations of all these physical factors is rendered evident by the fact that there are transitions from one structural type to the other (figs 10 and 11).

The protrusions, decreasing in number with progressing maturation, become gradually reduced to one or two foamy lobes, usually combined with serious mutilation of the cells (fig 13). Sometimes the peripheral ungranulated zone of the mature megakaryocyte shows the foamy transformation. In such instances the line of demarcation is absent (fig 13).

*Participation of the Nucleus*—Chromatin particles entering into the pseudopodia and suggesting that they share in the formation of the chromomere were never encountered. Even within the main cell body such particles are extremely rare, although the pluripolar mitosis so characteristic of the megakaryocyte would favor such occurrence. In a few instances (fig 14) budlike excrescences of the nuclei were limited to seriously injured cells. In figure 17, which is similar to the megakaryocyte depicted by Willi<sup>2b</sup> (fig 5 in his paper), the discontinuities and the serrated contour of the nuclear membrane suggest that the isolated extranuclear chromatin crumbs originated through mechanical dislocation. In all similar instances (figs 14, 15 and 16) the particles were never found within a protrusion and never showed any sign of further transformation. This apparent inactivity of the particles fits well with the behavior of an artificial product.

*Relations of Pseudopodia and True Thrombocytes*—Of all the protrusions, only those with clublike ends show greater resemblances



are produced in these sites. The correct interpretation of such misleading pictures presumes a detailed inquiry into the manner in which thrombocytes are liberated from the mature megakaryocytes and also into the possibility that one could be deluded by thrombocytes superimposed on, or apposed to, immature cells. These will be reported in another paper. Only a few points will be considered in the following comment.

#### COMMENT

From these observations the following significant facts emerge.

1. A distinct line of demarcation separates the bases of the excrescences from the main cell body.

2. The shape and the size of excrescences vary from filaments to lobes, and the number from a single protrusion to a corona of protrusions and eventually to a continuous envelope of the early megakaryocyte.

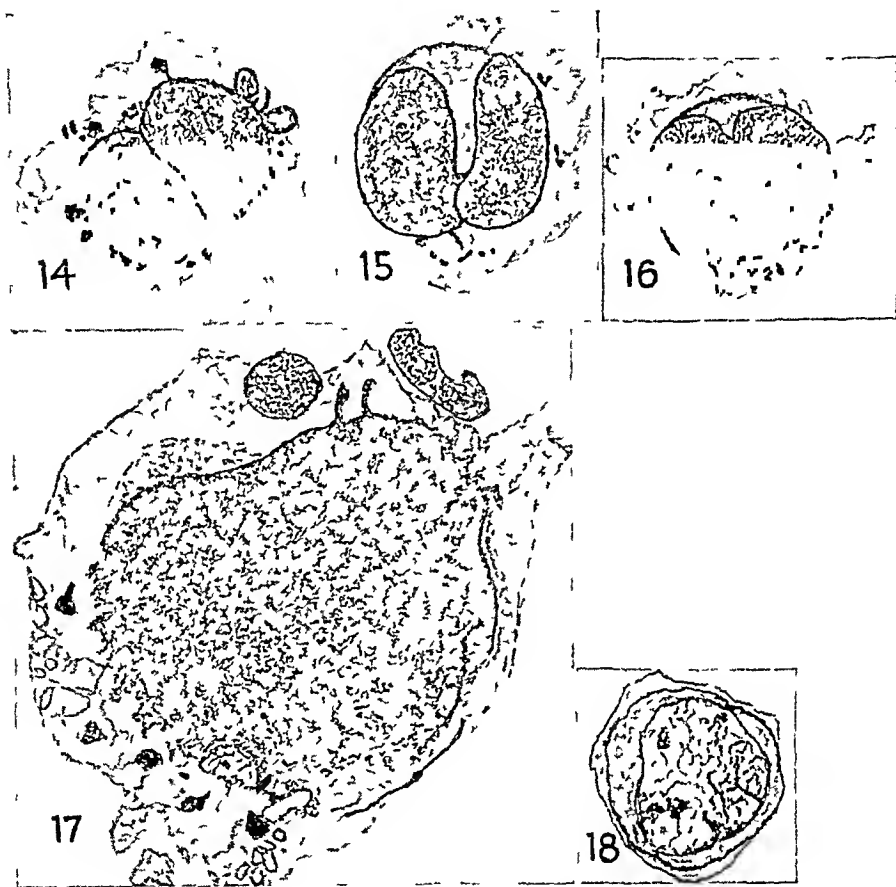
3. The protrusions exhibit structures identical with those seen in the intercellular sap of the smear, where they are formed according to the local variations of mechanical forces, the thickness of the film and the viscosity of the material.

4. Neither chromatin particles nor granules which do not stem from the functional area of granulopoiesis of the main cell body appear in the protrusions.

Consequently the existence of the line of demarcation does not permit one to interpret the excrescences as pseudopodia, since the latter as a product of ameboid activity obviously should stand in direct continuity with the cytoplasm of the cell body. This statement, together with the observations concerning the shape and the structural properties of the excrescences, qualifies them as the locally variable effects of the spreading and shearing forces acting on a viscous material that surrounds the megakaryocytes in their early stages. Therefore, the detached basophilic bodies carrying the features of the protrusions cannot be valued as the products of a vital development. They are artificially formed by the same forces as the protrusions themselves, without any relation to true thrombocytes.

The study of artefacts, though usually a sterile affair, yields here an interesting conclusion. An artefact produced by physical and chemical agencies is either inevitable in or accessory to the technical procedures to which the studied substrate was submitted. The constant occurrence of a special artefact stipulates that it must be related to certain properties of the substrate itself. The protrusions studied here appear on megakaryocytes of early developmental stages only and correspond to an artificial stretching and lacerating of a continuous layer. Therefore, this envelope represents a general property and either may be

to thrombocytes in their shape. They differ widely from the latter, however, in their texture. The possibility of a wrong interpretation is greater with the detached bodies, they show the structure of the protrusions, sometimes even their foamy appearance, but not the characteristics of the hyalomere of true thrombocytes. Granules in their interior stain basophilic (figs 2 and 3) but do not show the azure-like tint of a chromomere. Obviously, larger excrescences, especially the lobes, do not have to be considered



Figs 14 to 16—Megakaryoblasts showing nuclear buds and dislocated chromatin particles

Fig 17—Promegakaryocyte. The nuclear membrane has been artificially destroyed at some places. Note the dislocated chromatin particles in the cytoplasm

Fig 18—Early megakaryoblast with a narrow layer which enwraps nearly the entire cell body. From a section of marrow fixed in Zenker-formaldehyde solution, Giemsa stain

All the cells in figures 13 to 17 show clearly the mechanical deformation. Magnification, about 1,000

Unmistakable thrombocytes appearing between the protrusions or situated in the vicinity of, or on, the body of such a megakaryocyte probably are primarily responsible for the assumption that thrombocytes

itself of an artificial origin or may be a preformed though artificially deformed structure of the early megakaryocyte. The first hypothesis presumes that the mechanical forces expel from the cytoplasm a more fluid material, which thereafter is submitted to deformation. It is difficult to imagine that pressure and stretching may act so regularly on a cell of the size of a megakaryoblast and in such a way as to cause some material to be expelled in all directions around the cell. Neither would this hypothetic origin explain the constant and unbroken line of demarcation between the cell body and the corona of protrusions. The assumption that there is a preformed thin layer of a substance of lesser viscosity around the cell, stretched and disrupted by pressure and shearing, fits much better with all the manifest features of the protrusions and especially with the line of demarcation. Demonstration of the undisturbed layer in sections may permit a definite decision. However, as mentioned previously in these studies,<sup>3a</sup> the preserved three dimensional shape of the cell, the shrinking with fixation and embedding and the great difficulty of differentiating the youngest megakaryoblasts from other cells are responsible for the failure of this approach. Only after a long search may a cell like that in figure 18 be observed, suggesting the existence of such an extracellular layer. This layer should not be confused with the most outward of the three zones of cytoplasm of the mature megakaryocyte distinguished by Heidenhan<sup>5</sup> and confirmed by Frey<sup>6</sup>. These zones are intracellular, whereas the layer in question is superimposed on the cell contour.

In the first communication of this series<sup>3a</sup> it was pointed out that the hypothesis concerning a nuclear origin of the megakaryocytic granulation does not seem to be sufficiently substantiated. The statement of the artificial nature of the protrusions eliminates automatically any significance of chromatin particles which accidentally may have been dislocated into the excrescences. Thus any suggestion that thrombocytopoiesis has occurred in the ungranulated megakaryocyte becomes untenable. One can also dismiss the singular assertion of Hadorn<sup>7</sup> that the whole nucleus disintegrates into thrombocytes in the purpura caused by sedormid® (allylisopropylacetylcarbamide).

The observation that true thrombocytes are present in the vicinity of the protrusions or apparently within some of them or in the basophilic cell body itself accounts for the assumption of a precocious activity of these cells. As early as 1923 Naegeli<sup>8</sup> interpreted the

4 Schwarz, <sup>3a</sup> figure 1H

5 Heidenhan, M Arch f mikr Anat 43 423, 1894

6 Frey, H C Frankfurt Ztschr f Path 26 419, 1928

7 Hadorn, W Schweiz med Wchnschr 66 1273, 1936

8 Naegeli, O Blutkrankheiten und Blutdiagnostik, Berlin, Julius Springer,

accumulation of thrombocytes around nuclear fragments in the blood of patients with chronic myelosis as selective agglutination. This much discussed matter was settled by Undritz and Rothlin.<sup>9</sup> These authors compared smears prepared immediately and after a delay of one-half to one minute. Thrombocytes and nuclear fragments were well separated in the first smears but frequently found in aggregation in the later ones. Smears of rabbit marrow taken with and without citrate showed similar differences. Hemmeler<sup>10</sup> examined the marrow of patients with severe thrombopenic purpura with and without addition of normal blood. The admixed thrombocytes were found to gather frequently around the megakaryocytes, while in the other sample only single thrombocytes were seen to be attached to them. That thrombocytes have a capacity for selective agglutination is a well established fact.

My own extensive observations harmonize with the interpretation that superposition and apposition account for most of the deluding pictures. This is the more valid for figures 15 and 16 of Medlar<sup>2a</sup> and figure 4 of Juergens and Graupner,<sup>2c</sup> in which these strands of thrombocytes seem to emerge from the basophilic megakaryocytes with a smooth contour. Nevertheless, the observation of massive accumulation of thrombocytes around free megakaryocytic nuclei does not agree entirely with this explanation. A final decision may depend on whether the thrombocytes are liberated from the mature megakaryocyte singly or in strands at the periphery of the cell or whether they are set free by simultaneous disintegration of the entire cytoplasm. This hypothetic "explosive" disintegration was first advanced by Rohr and Koller<sup>2d</sup> as an additional mechanism besides the partial liberation of thrombocytes. It may, however, be emphasized that Sabin's<sup>11</sup> studies using the technic of supravital staining support the total disintegration as the more probable and regular process.

The elimination of the pseudopodia-like protrusions of the early megakaryocytes from any participation in the production of thrombocytes restores the notion of the unity of that process. Furthermore, it contradicts the developmental system of Juergens and Graupner,<sup>2c</sup> because no other way is known which would furnish thrombocyte-like bodies without a chromomere, assumed by these authors to be the juvenile stages. There is nothing else described in the literature or encountered in any of my observations which could be interpreted as a segregation of basophilic thrombocyte-like bodies lacking granulation. The arrangement of the granules of the megakaryocyte in the

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9 Undritz, E., and Rothlin, C. *Helvet med acta* **13** 595, 1946

10 Hemmeler, cited by Undritz and Rothlin.<sup>9</sup>

11 Sabin, F. R. *Bull Johns Hopkins Hosp* **34** 277, 1923

little groups of checkered pattern (*Felderung* of Seeliger<sup>12</sup>) provides the centers of disintegration of the mature cell. I have directed attention to the phenomenon of dissociation in which the nucleus of the megakaryocyte develops to normal maturity while the cytoplasm remains in the basophilic stage without a trace of a functional area. In no such instance could either a segregation of single thrombocyte-like bodies or a disintegration of the cytoplasm be observed. Therefore, it must be concluded that there is no justification for assuming either a juvenile nongranulated stage of the thrombocyte in physiologic conditions or a pathologic inhibition of that alleged development. The examples referred to by Juergens and Graupner<sup>2c</sup> do not, in my opinion, substantiate their system, since their juvenile stages cannot be traced back to megakaryocytes. Their illustrations must be interpreted as portraying artefacts according to the observations described in this paper. The source of these structures interpreted as "juvenile" or "pathologic" basophilic thrombocytes is to be looked for in other cells than the megakaryocyte. This was particularly emphasized by Castranuovo,<sup>13</sup> who traced these bodies back to any type of blood cells and called them "pseudo-platelets."

#### SUMMARY

In its early developmental stage the megakaryocyte is enveloped by a layer of a viscous material outside the cell contour. This envelope disappears with advancing maturation of the cell.

In the preparation of smears this thin layer is spread, lacerated and sheared off. Structures simulating ameboid pseudopodia result from the application of those forces. Such an origin of the pseudopodia is substantiated by the features of the excrescences. Their mechanical separation from the cell simulates the formation of thrombocytes. These pseudothrombocytes lack a chromomere. The hypothesis that a chromomere is formed in the protrusions is based on a wrong interpretation of appearances produced by the apposition and superposition of true thrombocytes.

This demonstration rules out any thrombocytopoietic activity of the basophilic early stages of the megakaryocyte. The uniformity of the production of thrombocytes, which is thus restricted to the granulated mature stages, is therefore restored.

Most of the structures described as "juvenile" or "pathologic" thrombocytes are pseudothrombocytes, which can be traced back to various basophilic, ungranulated stages of various types of blood cells.

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12 Seeliger, S. *Folia haemat* 29 23, 1923

13 Castranuovo, G. *Haematologica* 1 474, 1920

## ADRENAL PHEOCHROMOCYTOMA

J L PINNIGER, D M, M R C P (Lond)

AND

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FIFTY years ago (1899) pheochromocytoma of the medulla of the adrenal gland was recognized as a distinct entity at a postmortem examination by Robert. Nearly thirty more years passed before the tumor was diagnosed during life, and Charles Mayo's report of successful surgical removal quickly followed in 1927. Soon after this, in 1929, the epinephrine content of a tumor was estimated by Rabin. Since that time, further reports and reviews have been published which have added to knowledge of adrenal chromaffinoma or pheochromocytoma.

A case of pheochromocytoma of the medulla of the adrenal gland will now be described on account of the unusual size of the tumor and because studies have been made on its epinephrine content.

### REPORT OF A CASE

Miss M, aged 58, was in good health until a year before admission, when tachycardia and nocturnal dyspnea developed. Her physician regarded these symptoms as manifestations of cardiac failure, the cause of which he could not determine. The heart was not enlarged, no murmurs could be heard, and the blood pressure was not abnormal. For a week before admission the patient frequently wanted to pass urine but was unable to do so. On the day of admission her breathing became much more labored. At the time of examination she was semicomatose, and her skin felt cold and was of a mottled purple color, her temperature was 95.6 F. The pulse rate was 118, its rhythm was regular. The blood pressure reading was 105 systolic and 70 diastolic. The apex beat was in normal position, and no abnormal cardiac sounds were heard. The veins of the neck were considerably distended. The edge of the liver was just palpable. No subcutaneous edema could be detected. The respirations were increased in rate and depth. There was some diminution of the percussion note at the bases of the lungs. Crepitations, rales and rhonchi could be heard over the whole of the chest. In the left hypochondrium a tumor was felt, which was not tender. The pupils were dilated and of regular outline. No other physical signs were detected. Glycosuria was not demonstrated. The patient failed to respond to resuscitative measures and died twelve hours after admission.

*Postmortem Examination*—This was performed nine hours after death in mid-winter. On the left side of the abdomen a spheroid tumor was present, measuring 17 by 15 by 6 cm and weighing just over 1,200 Gm (fig 1). It lay above the

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From the Department of Pathology, Auckland Hospital

left kidney, which was depressed down to the rim of the pelvis. The spleen was stretched across the left side of the upper portion of the tumor, and the body and tail of the pancreas across its medial aspect. The tumor appeared to be covered completely by fibrous connective tissue. It felt partly cystic, partly firm. On section it was found to consist of purplish fleshy tissue, mottled gray in places, and was separated into lobules by strands of fibrous tissue. In places, chiefly centrally, orange masses occurred, which contained cystic spaces. The cut surface bulged considerably. No trace of normal adrenal cortex could be seen. A summary of the other postmortem observations follows. Small bilateral pleural effusions were present. Both lungs showed apical emphysema, congestion and at the bases subpleural collapse. The heart weighed 340 Gm. There was slight subendocardial fibrosis of the left ventricle. The left anterior descending coronary artery showed atheromatous narrowing and was calcified in places. The aorta was moderately atheromatous. The liver appeared a little congested. The thin-



Fig 1—Tumor of the medulla of the adrenal gland and the left kidney incised in the long axis

walled gallbladder was distended because of obstruction of the cystic duct by a faceted gallstone. There were many similar small gallstones in its cavity. There was slight congestion of the spleen and the kidneys. The right adrenal gland appeared natural. Death was considered to be due to acute cardiac failure arising as a result of pheochromocytoma of the medulla of the left adrenal gland.

*Histologic Examination*—Hematoxylin-eosin sections of formaldehyde-fixed material were first examined. The cells of the tumor were of variable size. They were polygonal in shape and in places formed syncytial masses. The cytoplasm was for the most part abundant, lightly to strongly eosinophilic and finely granular. The nuclei showed considerable variation in size and shape, some reaching giant proportions (fig 2A). A few of the cells contained more than one nucleus, one cell being observed with six. The staining of the nuclei was a little uneven. The nuclear membranes were distinct and the chromatin tended to be coarsely aggregated. In a proportion of cells nucleoli could be made out. Mitotic figures were

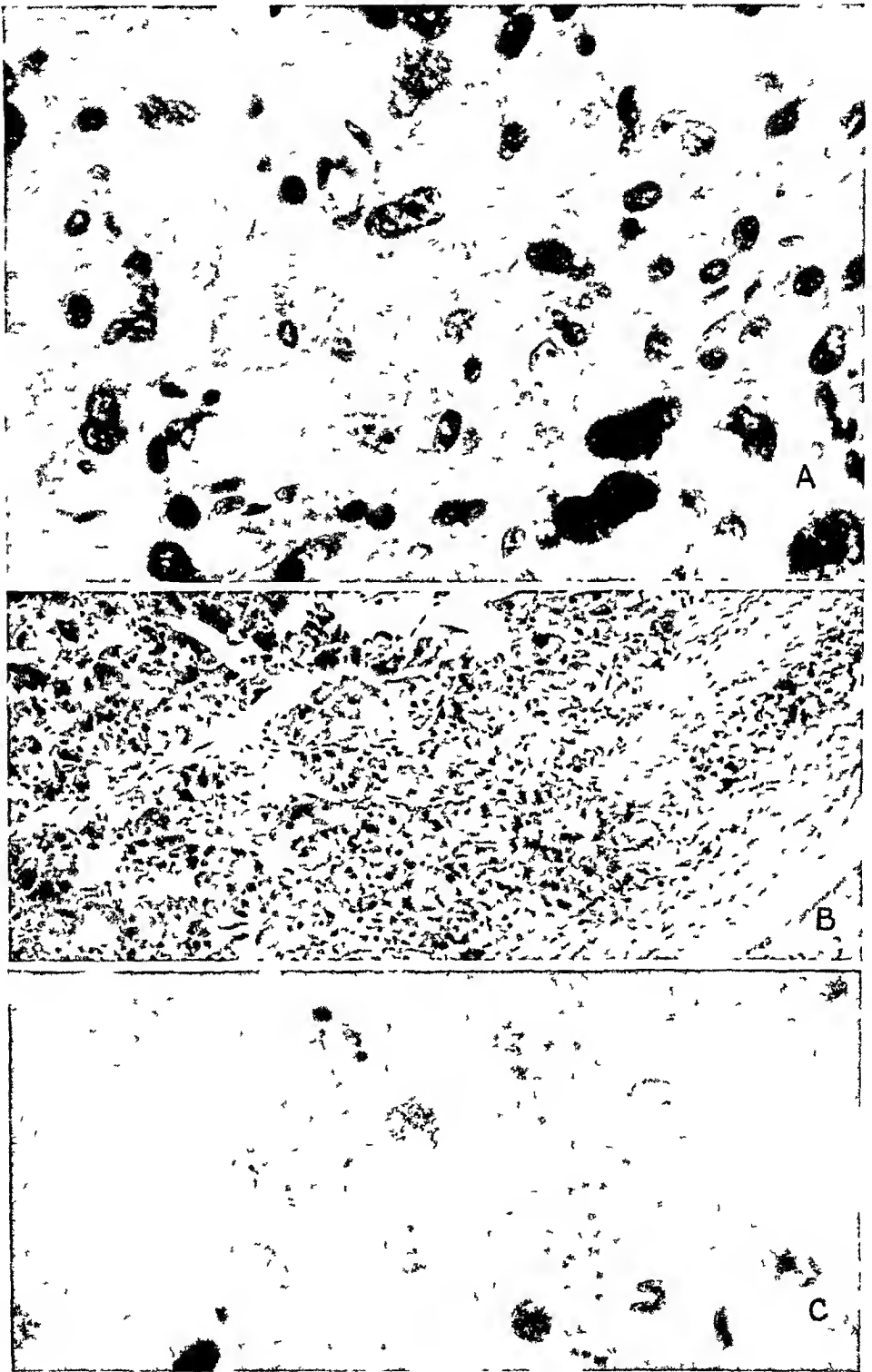


Fig 2—*A*, high power photomicrograph illustrating the pleomorphism of cells in some areas. Hematoxylin-eosin,  $\times 470$   
*B*, section of the tumor's edge, showing tumor cells infiltrating the fibrous capsule. Hematoxylin-eosin,  $\times 100$   
*C*, intracytoplasmic granules of epinephrine. Cramer's method,  $\times 1,300$



rare Basophil granules were seen in a few of the cells, being distributed usually at the periphery of the cytoplasm. The tumor tissue was intersected by fibrous trabeculae containing large blood vessels, otherwise the stroma was scanty, consisting of capillary blood vessels and patchily distributed thin strands of fibrous connective tissue. The tumor was surrounded by a fibrous capsule of variable thickness, which contained blood vessels, mostly of capillary size. Projections of tumor tissue extended into this capsule, and in places aggregates of cells lay apparently separated from the main mass (fig 2B). Within the tumor there were areas which showed varying amounts of degeneration. In some of these the cells had been replaced by a hyalinized loose fibrous connective tissue, in others, by an edematous and sometimes hemorrhagic matrix. Associated with one of the latter areas was a venule which showed a recently formed thrombus. A frozen section stained with scarlet red indicated that some of the tumor cells in this area had undergone fatty degeneration. Parts of the tumor showed a strong histologic resemblance to normal medullary tissue.

Stains for mucin, glycogen and alkaline phosphatase gave negative results. No nerve tissue was recognized in the substance of the tumor.

*Chromaffin Reaction*—When a piece of tumor tissue was immersed in a chromium salt fixative,<sup>1</sup> it became a rich brown within a few hours. A section of tissue fixed thus was stained after the method of Wiesel. Difficulty was found in producing any staining with the toluidine blue solution. Under oil immersion greenish brown granules of variable size could be seen lightly distributed throughout the sections. They lay usually in a paranuclear position. Another section fixed in the same way was stained by Schmorl's method for chromaffin cells. The cytoplasm of all the cells stained bluish green. In addition, globules of the same order as in Wiesel's preparation were present in the cytoplasm. These were of a more intense blue-green color.

It was further observed that the mass of tumor which was preserved in 4 per cent formaldehyde solution but otherwise exposed to the atmosphere changed from its initial purplish color to a deep rich brown in the course of two days. This became more intense on further standing, and the fixative also became stained this color. The tumor was later transferred to Pick III solution, and successive changes of this during the next four months were likewise stained brown, though with diminishing intensity.

#### THE DEMONSTRATION OF EPINEPHRINE

*Histologic Study*—The method of Cramer was applied to fat-free portions of the tumor tissue. The latter was fixed in osmic acid vapor, and some portions were afterward treated with turpentine. Black granules which were seen in the fixed and untreated specimens were unaltered by turpentine, in other words, these granules could be ascribed to epinephrine. They were usually in small clusters and visible only in a proportion of cells (fig 2C). Their size and number approximated those of the chromaffin granules seen in the sections stained by the methods of Wiesel and Schmorl.

*Chemical Studies of an Extract of the Tumor*—A portion of tumor weighing 44 Gm was immersed in 50 cc of 10 per cent trichloroacetic acid, cut up finely and allowed to stand in the ice chest overnight. The final volume was made up

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1 The formula of the chromium salt fixative used is as follows: 5 per cent potassium dichromate, 10 cc, 4 per cent formaldehyde solution, 20 cc, distilled water, 20 cc.

to 120 cc with distilled water, and the supernatant fluid was filtered. This filtrate was used for chemical studies and bioassay. It was found that the epinephrine in this filtrate was stable for at least six months.

Epinephrine was demonstrated in this extract by the following qualitative chemical tests. The extract gave a red color with the persulfate reagent of Ewing and a rose red color with iodine solution (Vulpian reaction), and it strongly reduced the uric acid reagent of Folin and Denis.

Three colorimetric methods were employed for the quantitative chemical estimation of the epinephrine present. A Coleman no. 11 spectrophotometer was used for this study. Pure crystalline epinephrine made up in hundredth-normal sulfuric acid was used for standard solutions.

#### 1. Method of Folin, Cannon and Denis<sup>2</sup>

Epinephrine may be estimated by its ability to reduce the uric acid reagent of Folin and Denis. Turbidity is prevented by adding urea. Details of the method used follow. To 1 cc of solution containing epinephrine at a concentration between 1/50,000 and 1/200,000 were added 0.5 cc of uric acid reagent, 1 cc of 40 per cent urea and 4 cc of saturated sodium carbonate solution. The blue color developed rapidly, was consistently reproducible and began to fade in five minutes.

By this method the epinephrine content of the tissue was estimated to be 27 mg per gram of tissue.

#### 2. Persulfate method first described by Ewins<sup>3</sup> and modified by Barker, Eastland and Evers<sup>4</sup>

Barker and associates described this as an almost specific chemical test for epinephrine in gland extracts, a red color being developed by the persulfate in the presence of epinephrine. They showed that maximum color developed in the presence of a chloride at a  $pH$  of 5.5 and that although color was produced more slowly in gland extracts than in pure epinephrine solutions, maximum color was produced in half an hour at 22°C.

Following their conditions, we have obtained complete color production by pure epinephrine solutions only after one and a half hours, while the color produced by gland extracts continued to deepen over a period of eight hours or more. The ultimate color contained a definite brown component, which in time produced a brown precipitate. Barker and associates stated that the persulfate reaction is catalyzed by traces of copper salts but that the color so produced is uncontrollable and unstable.

By carefully following the color production in the presence of copper ions we have obtained highly reproducible results with practically clear solutions. The technic was as follows:

To 4 cc of solution containing epinephrine in a concentration between 1/10,000 and 1/100,000 were added 4 cc of persulfate reagent and 2 drops of 1 per cent copper sulfate solution, and the  $pH$  of the mixture was adjusted with 4 per cent sodium hydroxide to between 5.0 and 5.5, according to an external methyl red indicator. The persulfate reagent was prepared as follows: Potassium persulfate 0.2 Gm, sodium chloride 1.0 Gm, disodium phosphate ( $Na_2HPO_4$ ) 0.1 Gm, monosodium phosphate ( $NaH_2PO_4 \cdot H_2O$ ) 1.0 Gm, and water to 100 cc. Readings of absorption at a wavelength of 500 millimicrons were made at frequent intervals until the maximum color had begun to fade.

2 Folin, O., Cannon, W. B., and Denis, W. *J. Biol. Chem.* **13**: 477, 1913.

3 Ewins, A. J. *J. Physiol.* **40**: 317, 1910.

4 Barker, J. H., Eastland, C. J., and Evers, N. *Biochem. J.* **26**: 2129, 1932.

It was found that the hydrogen ion concentration markedly influenced the rate of color production and fading but that the maximum intensity of color produced was not affected. Also, the color produced was proportional to the concentration of epinephrine present and obeyed Beer's Law. It seemed, therefore, that this modified procedure could be used to estimate reliably the amount of epinephrine in the gland extract.

By this method the epinephrine content of the tissue was found to be 3.8 mg per gram of tissue.

### 3 Method of Shaw<sup>5</sup>

Shaw described a method whereby reducing substances other than epinephrine were removed by aluminum hydroxide treatment. Glutathione is removed by aluminum hydroxide at  $pH$  4, while epinephrine remains in solution. Epinephrine is precipitated along with aluminum hydroxide at  $pH$  8 and is estimated by its reduction of a sensitive arsenomolybdic acid reagent. Shaw discovered that brief treatment with alkali enhances the reducing power of epinephrine and that this enhancement is specific for the side chain of epinephrine.

The aluminum hydroxide precipitations and alkali enhancement were carried out by us according to the aforementioned method. The concentrations of epinephrine used were adjusted to lie between 1:50,000 and 1:200,000, and the final blue solution was diluted to 50 cc with distilled water.

Contrary to the findings of Bloor and Bullen,<sup>6</sup> no loss of epinephrine occurred during the aluminum hydroxide treatments when solutions of pure epinephrine were used. Also, our gland extract lost no reducing power on full treatment with aluminum hydroxide. Although the degree of enhancement was not always reproducible, the special alkali treatment caused similar increases in color in both our gland extract and solutions of pure epinephrine.

The epinephrine content of the tumor was found by this method to be 2.7 mg per gram of tissue.

A pair of normal adrenal glands analyzed by these three methods was found to contain the following amounts of epinephrine:

|                                |        |
|--------------------------------|--------|
| (1) Folin, Cannon and Denis    | 4.0 mg |
| (2) Barker, Eastland and Evers | 2.9 mg |
| (3) Shaw                       | 2.4 mg |

Since the method of Folin, Cannon and Denis is affected by the other reducing substances normally present in the adrenal gland, it would be expected to give the highest values.

The discrepancies between the results obtained by the persulfate method and that of Shaw are similar for both tumor and normal gland. Since perfectly clear solutions were not obtained in the persulfate method, it is likely that the results obtained thereby were erroneously high. Shaw's method must therefore be considered as being the most suitable for the chemical estimation of the epinephrine of tissue extracts.

Since solutions of pure epinephrine and tumor extract behaved in identical manners in the procedure of Shaw, and as the values obtained for epinephrine content of the tumor by the three methods were approximately the same, it seems reasonable to conclude that 2.7 mg per gram of tumor represents the actual concentration of epinephrine and that no epinephrine-like compounds are present.

5 Shaw, F. H. *Biochem J* **32** 19, 1938

6 Bloor, W. R., and Bullen, S. S. *J Biol Chem* **138** 727, 1941

If it is assumed that epinephrine is distributed evenly throughout the tumor, the total amount of this substance is calculated to be 3.2 Gm

*Bioassays*—An aliquot of the tumor filtrate was sent to Mr F. N. Fastier, M. Sc., of the department of medicine of the University of Otago, who undertook to carry out bioassays by two methods. One of these made use of a rat hindquarter preparation.<sup>7</sup> Fluid was perfused through this, and the change in perfusion pressure brought about by the addition of tumor extract was compared with that produced by a standard epinephrine solution. This method gave the strength of the extract as 1 in 5,000. The other technique involved the inhibition of contraction of smooth muscle of rabbit intestine by epinephrine. The strength of the extract by this method was 1 in 3,500. These results of bioassays are equivalent to concentrations of 0.6 mg. and 0.9 mg. of epinephrine per gram of tumor tissue.

Mr. Fastier considered that the results might have been affected in some way by the solvent used.

#### COMMENT

The number of cases of pheochromocytoma which have been reported is expanding rapidly. In review of the world's literature Calkins and Howard<sup>8</sup> obtained a total of 176 such tumors. As a result of the clarification of the clinical picture, well summarized by Cahill,<sup>9</sup> an increasing number of cases are being diagnosed in life. The most striking modes of presentation are the result of paroxysmal hypertension. On occasions, however, the hypertension may be persistent,<sup>10</sup> and it has been reported malignant (i. e., rapidly progressive).<sup>11</sup> The diabetic features may be to the fore<sup>12</sup> and sometimes symptoms and signs suggest a diagnosis of thyrotoxicosis.<sup>13</sup>

However, as our case illustrates, there has been no good correlation between the severity of symptoms and the epinephrine content or the size of the tumor. Apart from the case of Borch-Johnson (cited by Biskind, Meyer and Beadner<sup>14</sup>) ours appears to be the heaviest tumor yet reported, being 200 Gm. in excess of that of Belt and Powell.<sup>15</sup> Except for the large quantity reported by the latter authors, the total epinephrine content of our tumor, 3.2 Gm., is apparently the greatest amount that has been found, and yet the disturbance

7 Fastier, F. N., and Smirk, F. H. *J. Pharmacol. & Exper. Therap.* **89**: 256, 1947.

8 Calkins, E., and Howard, J. E. *J. Clin. Endocrinol.* **7**: 475, 1947.

9 Cahill, G. F. *J. A. M. A.* **138**: 417, 1948.

10 Thorn, G. W., Hindle, J. A., and Sandmeyer, J. A. *Ann. Int. Med.* **21**: 122, 1944. Green, D. M. *J. A. M. A.* **131**: 1260, 1946.

11 Gutmann, D. *Brit. M. J.* **1**: 563, 1947.

12 (a) Duncan, L. E., Semans, J. H., and Howard, J. E. *Ann. Int. Med.* **20**: 815, 1944. (b) Goldner, M. G. *J. Clin. Endocrinol.* **7**: 716, 1947.

13 Cabot Case 32511, *New England J. Med.* **235**: 906, 1946.

14 Biskind, G. R., Meyer, M. A., and Beadner, S. A. *J. Clin. Endocrinol.* **1**: 113, 1941.

15 Belt, A. E., and Powell, T. O. *Surg., Gynec. & Obst.* **59**: 9, 1934.

to the patient was minimal. The patient did not complain of symptoms until a year before death, when symptoms developed, they consisted mainly of palpitations and shortness of breath, and until the terminal stage they were considerably less distressing than those which have occurred in other reported cases. The structure of the tumor suggests that growth had been taking place over a number of years. It is possible that a tolerance of the increasing amounts of epinephrine discharged into the blood stream gradually developed. Insensitivity to epinephrine, disappearing after removal of the tumor, has in fact been reported<sup>16</sup>

The macroscopic appearance of the tumor is similar to that of tumors of the same type described by Belt and Powell<sup>15</sup> and other authors. Histologically, too, the picture is similar to the descriptions given in other reported cases, e g, that of Edward<sup>17</sup>. As some tumors show a more regular cytologic pattern than others, they might be expected to secrete more epinephrine per unit mass than the latter. The cellular pleomorphism seen in this case (fig 2A) is not considered by Edward<sup>17</sup> to be an index of cancerous change, but this feature together with evidence of capsular infiltration (fig 2B) must be taken as indicating that our tumor was growing somewhat atypically. The amount of epinephrine per gram, 27 mg, was, in fact, considerably less than the amounts reported in a number of other cases<sup>18</sup>.

Basophilic cytoplasmic granulations such as those observed in some cells of our tumor have been noted by Mayock and Rose<sup>16</sup> and Duncan and co-workers<sup>12a</sup>. The latter commented on their resemblance to the Nissl granules of ganglion cells. No nerve elements could be recognized in our sections. In contrast to the findings of Muir,<sup>19</sup> no glycogen could be detected in the tumor cells. Fat globules could be demonstrated only in areas of degeneration. We were more fortunate than Edward<sup>17</sup> in being able to demonstrate epinephrine in the cytoplasm of some of the cells by the method of Cramer.

The chromaffin reaction was strongly positive. The term has been used in the literature to describe two features, the brown staining of cells treated with chromium salts and the intracytoplasmic brown granules demonstrated after chromium salt fixation. It has not been made clear whether the diffuse and the granular staining are in any way related. The former has been ascribed to the browning of intra-

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16 Mayock, R. L., and Rose, E. *Am J M Sc* **213** 324, 1947

17 Edward, D. G. *J Path & Bact* **45** 391, 1937

18 (a) Wells, A. H., and Boman, P. G. *J A M A* **109** 1176, 1937 (b) Spalding, J. M. K. *Brit M J* **1** 565, 1947. Belt and Powell<sup>15</sup>

19 Muir, R. *Textbook of Pathology*, ed 5, London, Edward Arnold & Co., 1941, p 965

cellular epinephrine,<sup>20</sup> whereas other authors<sup>18a</sup> have considered that the granules appear when the chromium salts have been reduced by the epinephrine to an insoluble peroxide of chromium

Our tumor became stained a deep brown quickly on addition of potassium dichromate, but it also showed the same color change, more slowly, after being exposed to air. The numbers of chromaffin granules which we were able to show by the methods of Wiesel and Schmorl were of the same order as that of the epinephrine globules shown by the method of Cramer

It seems likely, therefore, that the potassium dichromate merely greatly accelerates the oxidizing of epinephrine to a brown compound which will appear in any case at a slower rate in the atmosphere. That chromium enters into the composition of the granules is suggested by the following experiment. When equal parts of 0.1 per cent epinephrine solution and potassium dichromate fixative were mixed, the solution first became red and then dark brown, and in a few minutes a brown precipitate formed. After the latter had stood for a few days, it was centrifuged and washed with distilled water. On analysis it was found to contain both organic matter and chromium

#### SUMMARY

A case in which pheochromocytoma of the medulla of the adrenal gland was diagnosed at postmortem examination is described. The tumor was large, weighing 1,200 Gm. Chemical and biologic assays of epinephrine were made on extracts of this tumor. The relative merits of the methods for the chemical estimation of epinephrine were investigated, and it was found that the technic of Shaw was the most suitable for this purpose. By it the total amount of epinephrine in the tumor was calculated to be 3.2 Gm. The chromaffin reaction is discussed

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20 Carleton, H. M., and Leach, E. H. *Histological Technique*, ed. 2, London, Oxford University Press, 1947, p. 289

# GENIC FACTORS IN VISCERAL ASYMMETRY AND IN THE DEVELOPMENT AND PATHOLOGIC CHANGES OF LUNGS, HEART AND ABDOMINAL ORGANS

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**S**ITUS inversus viscerum is one of the most fascinating of the anomalies of man. To judge from the number of papers on the subject, interest in it is equaled only by that in the transposition of the great vessels, the puzzling anomaly correlated to situs inversus. The intention of the present paper is to give further insight into these problems on the basis of a study of the genetics and the developmental genetics of situs inversus.

Situs inversus is no simple alternative to the normal situs. It is the most complicated among anomalies, concerning not only the situs, but all details of structure. There are a great many studies of the anatomy of situs inversus, culminating in the papers of Pernkopf (1937).

The problem of situs inversus is a part of the problem of symmetry and has called on the interest of medical men, anatomists and other scientists in the different fields of biology. The literature on situs inversus and related topics is immense, therefore, and in this brief survey I can deal only with a few previous studies that are essential to an appreciation of the problems of the present paper.

Spemann and his pupils found the anomaly in about one half of the twins which were derived from the right halves of eggs divided mechanically in different stages of development. They also produced inversion of the heart by rotating pieces of the roof of the primitive gut. Komai (1938) found in salmon a high correlation between situs inversus, small size and malformations which occurred in single individuals as well as in separate and in conjoined twins, presumably due to the environmental factors in the fish hatcheries.

Situs inversus was first found as a genetic variation in experimental animals in 1948, when Tihen, Charles and Sippel published their observation of situs inversus as caused by a recessive gene in mice. As in man so in these animals there were several associated anomalies, particularly hydrocephalus. They found 29 affected individuals and 227 normal individuals among the offspring of heterozygotes, about one

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half of the expected number. They suggested that this may be due to prenatal selection or to some variation of the phenotype. Nothing is known as yet about the hereditary and developmental relationship between situs inversus and the associated anomalies in mice.

Several cases are on record showing that the condition may be hereditary in man. Mattison observed the anomaly in mother and daughter, while Pernkopf (1937) found complete situs inversus in a father and partial situs inversus in his child. Mattison examined 448 members of the families of 4 patients without finding it in any and Gutzeit and Lehmann had the same experience in an extensive examination of the families of 3 patients.

As far as I know, 29 families are on record with more than 1 sibling affected—7 with 3 siblings affected, 22 with 2 affected (Cockayne, 1938, Gansslen, Lamprecht and Werner). Two of the families in which 3 were affected and 2 of those in which 2 were affected were reported from Norway, by Frolich, Torgersen (2 cases, 1946) and Natvig.

The only investigation based on a large number of cases is Cockayne's (1938). His paper represents the most important approach to the genetics of situs inversus. Cockayne found 58 affected and 90 normal persons in 52 reliable sibships. This gives, according to Cockayne's calculation, a ratio of affected to normal of 1:1.7, which represents, as far as I can see, the ratio of the index cases to the normal siblings. In a review of the literature he found a ratio of affected to normal of 1:2.9 in 119 sibships with 137 affected and 402 normal siblings. Cockayne concluded, therefore, that the anomaly is determined by a single recessive gene. He interpreted the associated anomalies as expressions of a manifold effect of this gene. Gutzeit and Lehmann admitted the possibility that there was dominant inheritance in some cases.

As far as I know, situs inversus has been observed 12 times in monozygotic twins, 6 times concordant and 6 times discordant (Cockayne, 1938, Gansslen, Lamprecht and Werner, Kean, Helweg-Larsen). According to Cockayne (1938), discordant situs inversus has been observed once in dizygotic twins.

Mattison made an extensive investigation of the occurrence of twins in the families of persons with situs inversus and found an insignificantly higher percentage than in the population. There are on record a few cases indicating a relationship between twinning and situs inversus. Doolittle reported a case in which a probable isolated dextrocardia occurred in a pair of dizygotic twins, the father of whom was a dizygotic twin with dextrocardia. There were many twins in the family on both the paternal and the maternal side. Helweg-Larsen reported that there were several twins in the family in his case.



Joyce asked in the *British Medical Journal* whether it depended on chance that he had observed 2 cases of situs inversus in which there were surprisingly many twins in the families, both parents being twins in 1 case. In Reinhardt's case (1912) the mother of the twins with situs inversus was a twin and she and their father were first cousins. Most remarkable are the families in which parents who are twins have twins showing situs inversus.

Cockayne (1938) expressed the opinion that the frequent concordance noted in one egg twins is evidence of heredity. In 1939, in view of the observation of a discordant monozygotic pair of twins, he admitted the possibility that a mirror image effect occurs in cases of late division. This possibility is confirmed by the fact that all the cases of isolated dextrocardia on record are discordant, 3 of the patients were separate twins and 2 were conjoined twins. In the discordant cases of Dubreuil-Chambardel, Cockayne (1939) and Helweg-Larsen there is some dissimilarity of the twins, the first pair showing mirror image harelip and the second mirror image asymmetries indicating a late division. These twins may have narrowly escaped being conjoined twins.

Cockayne found first cousin marriages in 11 per cent of 53 cases, and he emphasized that even 6 per cent would be a convincing proof that the condition is determined by a single recessive gene. Cockayne originally suggested the idea of recessive inheritance in the discussion concerning the familial case of Feldman, in which the parents were first cousins.

A supposition of a particular mechanism of inheritance increases the risk of selection. I have found first cousin marriages in only 2 of the 29 familial cases of situs inversus in the literature (Ochsenius and Feldman). There may be some more but I have not had an opportunity to study all the original papers.

Situs inversus may be associated with bronchiectasis and nasal polyps (Kartagener, Olsen, Torgersen, 1947). There is good evidence that bronchiectasis may be inherited. The most reasonable interpretation of the data is that the genes causing bronchiectasis behave in the same way as genes showing incomplete dominance. The syndrome has been observed 3 times in father and child, 10 times in siblings, 4 times in both of a pair of monozygotic twins and 2 times in one of a pair of dizygotic twins (Kartagener, Diehl). Familial occurrence of situs inversus and bronchiectasis has been observed by Cockayne (1938). In a sibship of 5 bronchiectasis was found in one sib and situs inversus in another. Jerman (cited by Diehl) observed situs inversus in a mother and bronchiectasis and chronic ethmoiditis in her son. Kartagener reported situs inversus occurring in one brother and situs inversus and bronchiectasis in another. These observations are

more indicative of genes causing bronchiectasis as a primary effect and situs inversus as an expression of a pleiotropic effect than of genes causing situs inversus as a primary effect and bronchiectasis as a secondary effect

Adams and Churchill had examined the patients of the Massachusetts General Hospital since the year 1886, numbering in all 232,112. Bronchiectasis occurred in 0.36 per cent, situs inversus, in 0.01 per cent. Bronchiectasis occurred in 21.7 per cent of the persons showing situs inversus, situs inversus occurred in 0.7 per cent of the persons showing bronchiectasis.

The previous observations of the frequency of bronchiectasis in persons with situs inversus are set forth in table 1.

The agreement among the authors as to the frequency of situs inversus in cases of bronchiectasis and of bronchiectasis in cases of situs

TABLE 1—Recorded Instances of Situs Inversus Complicated with Bronchiectasis

|                            |  | Patients | Per Cent |
|----------------------------|--|----------|----------|
| Horlacher (cited by Diehl) | Situs inversus   | 30       |          |
|                            | Bronchiectasis with situs inversus                             | 7        | 23.3     |
| Olsen                      | Dextrocardia   | 85       |          |
|                            | Bronchiectasis with dextrocardia, 10 of whom had nasal polyps  | 14       |          |
|                            | Nasal polyps with dextrocardia without bronchiectasis          | 4        | 21.2     |
| Torgersen (1947)           | Situs inversus   | 129      |          |
|                            | Bronchiectasis with situs inversus, 5 of whom had nasal polyps | 10       |          |
|                            | Nasal polyps with situs inversus without bronchiectasis        | 6        |          |
|                            | Severe bronchitis or frequent pneumonia with situs inversus    | 18       | 22.4     |

inversus is evidence of the validity of the observations. Adams and Churchill discussed the difficulties in the making of the clinical diagnosis and in the separating of the congenital from the acquired defects. It is impossible to make a clinical differential diagnosis between severe chronic bronchitis, recurrent pneumonia and bronchiectasis. They stress the difficulty of establishing a correlation between these and such common diseases as sinusitis. One has also to consider that bronchiectasis is much more common than is generally assumed. Among the chronic diseases of the lungs it is second in importance only to tuberculosis.

Why do some people have bronchiectasis after one of the common nontuberculous diseases of the lungs and others not? This as a question of both heredity and environment. It is impossible to draw a sharp line between these two main factors in pathology as well as in genetics. Roberts mentions a family with many cases of sinusitis. He is inclined to mean that the familial sinusitis is due to particular genes and that it is, in consequence, etiologically different from the corresponding common disease.

In another preliminary paper (Torgersen, 1947) mention has been made of the rather frequent anomalies of the spine in persons with situs inversus

Situs inversus is often complicated with malformation of the heart. This combination is so common that anatomic and etiologic studies of situs inversus and of congenital heart disease have to be based on this correlation. Spitzer, Aschoff and Pernkopf (1937) stressed the importance of this combination.

As may be seen, a study of situs inversus has to include the genetics and the pathology of common diseases. This makes the analysis of the material difficult. However, the rarity of situs inversus facilitates the establishment of a correlation between it and even rather common diseases. The motive of the present investigation has been not only an academic interest in situs inversus but also a practical interest from this particular point of view in the causation of these common diseases.

TABLE 2—Collection of Cases

|   |   |           |
|---|---|-----------|
| Mass roentgenography  | Number of persons   | 1,000,000 |
|   | Confirmed cases of <i>situs inversus</i>  | 104       |
|   | Frequency 0.01 per cent $\pm 0.0003$  |           |
| Hospitals and physicians                                    | Cases of <i>situs inversus</i> , 10 of which are already included in the 104 cases from the mass roentgenography series | 98        |
| Sum total (104 + 98)  |   | 192       |
| Expected number of cases of <i>situs inversus</i> in Norway |   | 300       |
| Not diagnosed, about  |   | 100       |
| Family material   | Number of families  | 161       |
|   | Number of cases in these families   | 168       |

## OWN INVESTIGATIONS

The data and an evaluation of the data are presented in this chapter. I have added some parallel observations from the literature, both from that concerned with human pathology and genetics and from that based on experimental embryology and genetics. In the last chapter I will discuss the bearing of the data on the problem of situs inversus and the development of the viscera and their predisposition to the diseases referred to in the foregoing pages.

My plan was to collect as many cases as possible from a small and geographically limited population. This decreases the risk of selection and also the risk of not detecting cases in sibships. As will be seen from table 2, about one half of the cases have been detected in a mass roentgenography series, the other half have been reported to me by physicians and from hospitals and institutions of public health. It may seem simple to diagnose situs inversus in mass roentgenography. There are some difficulties, however, which are familiar to people used to the technic. I have therefore included only cases in which there is no doubt about the diagnosis and cases in which the diagnosis has been confirmed by ordinary roentgen examination.

None of the numbers in the foregoing table can be regarded as quite exact. However, the errors are significant. Thus, situs inversus occurs in the population at a rate probably somewhat above 0.01 per cent. This corresponds to a total

number of 300 persons with situs inversus, about 66 per cent of whom have been detected. This makes it improbable that a significant number of cases, if any, are to be found in the sibships and among the parents of the persons with situs inversus. It is hardly possible to examine all the siblings personally, but there has been a great chance of detecting the anomaly in these families, which have been most interested in the present study, and most people in Norway have been examined by physicians, a great many also by roentgenologists. In two counties 85 per cent of all persons above 15 years of age have been examined by mass roentgenography. No familial case was detected in the examination of 139,596 persons, 17 of whom had situs inversus.

TABLE 3—*Cases of Situs Inversus in Two Counties in Which Examinations Were Made*

|                                     |                            |                   |                  |
|-------------------------------------|----------------------------|-------------------|------------------|
| Oestfold                            | Number of persons examined | 72,690            | (84.17 per cent) |
|                                     | Number with situs inversus | 5                 | (0.007 per cent) |
| Opland                              | Number of persons examined | 66,906            | (86.95 per cent) |
|                                     | Number with situs inversus | 12                | (0.02 per cent)  |
| Difference between the two counties |                            | 0.013 $\pm$ 0.008 |                  |

TABLE 4—*Discrepancy Between Actual and Expected Incidence*

|          | Actual Number | Expected Number | d   | d <sup>2</sup> | d <sup>2</sup> /e    |
|----------|---------------|-----------------|-----|----------------|----------------------|
| Oestfold | 5             | 8.8             | 3.8 | 14.44          | 1.6                  |
| Opland   | 12            | 8.1             | 3.9 | 15.21          | 1.9                  |
|          |               |                 |     |                | X <sup>2</sup> = 8.5 |
|          |               |                 |     |                | p = 0.05             |

TABLE 5—*Differences Between the Two Counties in Which Examinations Were Made*

|  | Oestfold | Opland |
|--|----------|--------|
| Density of population                        |          |        |
| Living in rural districts, per cent          | 31.5     | 5.4    |
| Living in cities, per cent                   | 30       | 7      |
| Ratio first births to later births 1920/1930 | 28/72    | 25/75  |
| Ratio first births to later births 1931/1941 | 42/58    | 35/65  |
| Marriage frequency, per cent                 | 6.42     | 5.07   |

The population in these two counties has been examined to such an extent that one gets some information about geographic variation, as shown in table 3.

An X<sup>2</sup> analysis considering the expected number of cases of situs inversus if the frequency had been the same in the two counties gives the result shown in table 4.

The difference probably depends on some other factor than chance, therefore. The two counties represent two extremes in the Norwegian population. The difference may be seen better from table 5 than from a description.

The urbanization of the population is more advanced in Østfold than in Opland, where the people to a great extent live in valleys separated by the high mountains. The proportion of first births is greater in Østfold and so is the marriage frequency. There are relatively many, but small, families, and the age of the mothers at birth of the children will be lower because the few children are born

in the first years of marriage. The frequency of the birth of twins as seen from table 6 shows a difference in the two counties. It is hardly possible to decide on the basis of the present knowledge whether this discrepancy between a district with many isolated groups and a district with far advanced urbanization is due to inbreeding and genic factors or to the breeding habits of the population, expressing itself in the age of the mothers at birth, or to unknown factors.

In these counties 97 per cent of the births have been registered. It is improbable that there are so many twins in the remaining 3 per cent that this will influence the frequency in the statistics.

The correspondence between the occurrence of situs inversus and that of twin births may be evidence of a causative relationship. However, both may be due to independent factors tending to be relatively common in isolated groups.

The size of the population is 3,000,000. Supposing the frequency of twin births to be about 14 per cent, one should expect about 60,000 twins in the country, considering the high infant mortality, about 45,000 dizygotic and 15,000 monozygotic twins. Supposing a frequency of situs inversus of 0.1 per cent, one would expect 15 monozygotic twins with situs inversus and 45 dizygotic twins with the anomaly. I have actually found 2 possibly monozygotic twins, as well as 2 dizygotic twins, with situs inversus—the latter 2, surprisingly, both

TABLE 6—Occurrence of Twins in the Two Counties Examined

|  |           |             |
|--|-----------|-------------|
| In Oestfold  | 1920-1930 | 15 per cent |
|  | 1931-1941 | 11 per cent |
| In Opland  | 1920-1930 | 16 per cent |
|  | 1931-1941 | 15 per cent |
| Difference of Opland and Oestfold averages $155 - 13 = 0.25 \pm 0.033$ |           |             |
| Oestfold 1920-1930 and 1931-1941 percentages = $0.4 \pm 0.3$           |           |             |

in the same pair. One of the monozygotic twins and one of the dizygotic twins with situs inversus was detected in the mass roentgenography series. Therefore, it is highly improbable that there should exist more twins with situs inversus in the country.

In one of the cases in which a probable monozygotic pair of twins was involved, the twin sister died at the age of 6 years. In the other case the twin sister died at the age of 1 month, and the statement of the mother that the twins showed great similarity does not mean much. There is no reasonable doubt that the third case concerned a pair of dizygotic twins (Torgersen, 1949).

Partial inversion of the particular type observed in 1 of these dizygotic twins occurred in 3 persons included in the present study, their number corresponding to a frequency of 0.0001 per cent. The probability that in this case the coincidence of twinning and situs inversus is due to chance is small. The chance that a sibling will show situs inversus, excluding the index cases, is about 1 per cent. The most reasonable assumption is that the inversion is partly due to some environmental factor, possibly causing a division in both of a dizygotic pair of twins. In other words, these twins may be survivors of quadruplets. The frequent occurrence of twin births in the family of the father is remarkable, considering the similar observation of Doolittle referred to in the introduction. The questions of the hereditary mechanism in twinning and the influence of the father are still unanswered.

Pernkopf (1937) was inclined to explain situs inversus as depending on some abnormality of the sperm, owing to his observation of visceral inversion

in father and child It must be admitted that the observation of concordant situs inversus in dizygotic twins in a family with many twins on the paternal side may support his view

There is no significant increase of the frequency of twin births in the siblings I have reliable data about twins among the parents and grandparents in 30 families In 2 cases one of the grandparents was a twin This is not surprising However, it is remarkable that in both these cases the patient with situs inversus was a twin, in one a monozygotic twin and in one a pair of dizygotic twins In 1 case the mother was a twin and she and the father were first cousins These observations in connection with the corresponding observations mentioned in the introduction are good evidence that there is a correlation between twinning and situs inversus in a few cases

The data indicate that the frequency of monozygotic twins in cases of situs inversus does not deviate greatly from their frequency in the population This

TABLE 7—Comparison of Ages of Mothers at Bnth of Children

|  |                             |
|--|-----------------------------|
| The ages of mothers known in               | 164 cases of situs inversus |
| The ages of mothers known in               | 157 index cases             |
| The ages of mothers known in               | 7 secondary cases           |
| Mean age of mothers in cases (5,184 — 164) | 31.6                        |
| Mean age of mothers at birth generally     | 30.5                        |
| Difference                                 | $1.1 \pm 0.46$              |

TABLE 8—Actual and Expected Numbers of Mothers in Age Groups

| Age of Mothers | Percentage in Population | Actual Number | Expected Number | d    | d-     | d <sup>2</sup> /e                 |
|----------------|--------------------------|---------------|-----------------|------|--------|-----------------------------------|
| 15-19          | 1.5                      | 2             | 2.5             | 12.3 | 151.29 | 4.68                              |
| 20-24          | 18.2                     | 18            | 29.8            | 0.6  | 0.86   | 0.01                              |
| 25-29          | 28.4                     | 46            | 46.6            | 3.0  | 9.0    | 0.23                              |
| 30-34          | 23.8                     | 42            | 39              | 6.8  | 46.24  | 1.58                              |
| 35-39          | 17.8                     | 36            | 29.2            | 3.1  | 9.61   | 0.57                              |
| 40-44          | 8.9                      | 15            | 14.6            |      |        |                                   |
| 45             | 1.4                      | 5             | 2.3             |      |        |                                   |
|                |                          | 164           | 164             |      |        | $X^2 = 7.07$<br>$p = 0.10 - 0.20$ |

may be interpreted as evidence that in man monozygotic twinning plays no great part in the production of situs inversus The numbers further indicate that the frequency of situs inversus in dizygotic twins is low, a supposition which is confirmed by the observations available in the literature

In table 7 I have compared the age of mothers at the birth of children showing situs inversus with the age of mothers at the birth of children in the country generally The numbers are small, because of the rarity of the anomaly, and difficult to evaluate, therefore

In table 8 is seen the deviation between the actual and the expected numbers of mothers of children with situs inversus in the different age groups

The difference between the age of mothers at birth of children with situs inversus and the age of mothers at birth of children generally is somewhat below 3 times the standard error The X<sup>2</sup> analysis does not show any convincing evidence of a high age of the mothers However, the consistent deviation in all age groups indicates a high age of the mothers

The possibly high age of the mothers is due mainly to the 54 cases of situs inversus combined with bronchiectasis or congenital heart disease (table 9)

In table 9, also, the consistency of the deviation in all groups is conspicuous

I have further analyzed the material to see if the order of birth has any influence. The child with situs inversus was the only child in 13 families. With these families excluded, it was the first-born in 29 families, the last-born in 34 families. Again, 440 children were born before and 334 children after the child with situs inversus. This difference is significant. As far as I can see there is no selective factor favoring the last orders of birth. According to Penrose (1934), there is reason to assume a selective factor favoring the first orders. In the families with more than two children in all, 20 per cent of the 270 first-born and last-born showed situs inversus, against 14 per cent of the 638 in the middle of the sibships. The difference is  $6 \pm 2.29$ . It is thus probable that the distribution of the children with situs inversus in the sibships is not due to chance alone.

TABLE 9—*Numbers of Mothers in Different Age Groups Who Gave Birth to Children in Whom Situs Inversus Was Complicated with Bronchiectasis or with Malformation of the Heart*

| Age of Mothers | Bronchiectasis | Hearing Malformation | Sum Total | Expected e | Deviation d | d <sup>2</sup> | d <sup>2</sup> /e | Familial Cases |
|----------------|----------------|----------------------|-----------|------------|-------------|----------------|-------------------|----------------|
| 15-19          | 0              | 0                    | 0         | 11         | 7           | 49             | 4.45              | 2              |
| 20-24          | 3              | 1                    | 4         |            |             |                |                   |                |
| 25-29          | 9              | 1                    | 10        |            |             |                |                   |                |
| 30-34          | 12             | 3                    | 15        |            |             |                |                   |                |
| 35-39          | 13             | 4                    | 17        | 6          | 2           | 4              | 0.67              | 1              |
| 40-44          | 4              | 1                    | 5         |            |             |                |                   |                |
| 45-            | 3              | 0                    | 3         |            |             |                |                   |                |

$$\chi^2 = 11.99$$

$$p = 0.02$$

The data, taken as a whole, are rather good evidence of an influence of the age of the mothers and a corresponding influence of the order of birth due to cases complicated with bronchiectasis and congenital heart disease.

According to Roberts, it is known that the age of mothers is high at the birth of children with congenital heart disease. Nothing is known about the age of mothers at the birth of children with bronchiectasis.

The lung-nose syndrome occurred in 4 of the 5 familial cases. In all these families except 1 the mothers were particularly old at the birth of the children with situs inversus. In the exception in which the mother was young there was evidence that the syndrome occurred in both parents. This may indicate that the influence of the age of the mothers in these cases concerns the manifestation of the genes.

The most reasonable conclusion is that the anomalies which are mostly influenced by the age of the mothers are the primary ones, the others, such as situs inversus, being expressions of the manifold effect of the genes. A parallel may be seen in cases of mongolism. The disturbance of the development of the heart is hardly the primary effect of the genic and nongenic factors, including the age of the mothers but is secondary to the general disturbance of growth.

In 1 family the stature of the mother and 8 children was at the lower limit of normal. The last-born showed situs inversus and a congenital heart disease, probably the tetralogy of Fallot. The child next to him showed symptoms of idiocy. A sister showed retardation of the maturation of the bones and a short

middle phalanx of the fifth finger The symptoms of mongolism are thus found in different members of the family The father was of average stature, and the relative parts played by heredity and environment in this case are obscure The living conditions of the family were poor, and the mother had given birth to 8 children by the time she was 32 years old There is nothing remarkable about the physique of most of the persons with situs inversus Constitutional anomalies have been recorded in some cases (Torgersen, 1948)

The average size of the 161 sibships was 5.9 I have compared the actual and the expected size of the sibships, supposing that the size of the sibships had been the same in cases of situs inversus as in the general population in the same years There are significantly more large families in the cases of situs inversus However, this is most probably due to the selection of large families in surveys in which only those families are recorded which contain affected persons (Penrose, 1934) The average size of the families is not surprising when one considers that a great many of the marriages took place about the year 1900 and that the average size of a Norwegian sibship in 1920, after twenty years of marriage, was 5.03

In the total material 5 per cent of the children died before 1 year of age The corresponding percentage in the population is 4.5 per cent In the 40 families in which the lung-nose syndrome occurred 12 per cent of the 200 children died before 1 year of age The difference between the percentage in this group and that in the total material is  $7 \pm 3$  This may indicate a somewhat increased mortality in this group As there is no sign of an increased mortality of infants in the other cases, it is reasonable to assume that the possibly increased mortality of infants of the group showing the lung-nose syndrome is due to a predisposition to diseases of the respiratory tract A particular observation increases the probability of an increased mortality of infants in these cases It is not included in the foregoing calculation A woman with situs inversus was the last-born of 13 children, 10 of whom died before they were 1 year of age One of her sisters had 13 children, 10 of whom died before they were 1 year of age In another case the parents had a child with situs inversus, six lumbar vertebrae and spina bifida occulta The mother gave birth a year later to a child with spina bifida aperta and had after that two abortions In a third case a stillbirth occurred after eleven years of involuntary sterility following the birth of a child, the first-born, with situs inversus In 2 more cases three stillbirths occurred besides the birth of a child with situs inversus In one of the familial cases there was good reason to assume that sterility was present in the sibship itself, 5, possibly 6, siblings in a sibship of 8 being sterile This family showed the lung-nose syndrome, the father and 4 children being affected The parents were second cousins An analysis of the pedigree does not indicate a linkage between genes causing these peculiarities

The probably low frequency of situs inversus in dizygotic twins may according to Weinberg (1907), be a sign of prenatal selection In some cases only one of a pair of dizygotic twins will have the lethal factor The other will pass as not having been a twin, an event tending to decrease the number of dizygotic twins The significance of these data is difficult to estimate A prenatal mortality sufficient to explain the deficiency of affected persons when one assumes a single recessive gene presumes an improbably high mutation rate to compensate the corresponding loss of genes

According to Roberts, there is no agreement among the statisticians as to what procedure is the best in order to avoid a selection of affected persons in a study of a character which is supposed to be due to a recessive gene As



all the cases have been detected by an index case, I have excluded these cases as selected. The 2 familial cases which had been recorded previously should also have been excluded, thus making the number of secondary cases still lower. The number of index cases is 161, the numbers of affected and normal siblings are 7 and 777 respectively, giving a percentage of affected siblings of 0.9. The great deviation from the expected ratio in recessive inheritance is evident also from the fact that the index case is that of the only affected person in 156 families. It is also surprising that so few familial cases are on record. There is no reason to assume that this is due only to nondetection if one considers the extensive mass roentgenography carried on in many countries in recent years.

As the frequency in the population is about 0.01 per cent, the notion that the secondary cases are due to chance can be excluded. Murphy found that congenital malformations will occur twenty-five times as frequently as in the whole population in families in which the parents already have a malformed child. Situs inversus will occur one hundred times as frequently in families in which the parents have a child with situs inversus. This indicates a relatively strong influence of the genes in situs inversus. The number of index cases in the group of familial cases was 5, and the numbers of affected and normal siblings were 7 and 21 respectively. The fit to the mendelian ratio in these cases is probably not due to chance. The lung-nose syndrome occurred in 4 of these families—in both parents in 2 families, in one of the parents in 1 family. As mentioned, Kartagener reported the syndrome observed in a familial case. Lopez (1945) saw the syndrome in 3 siblings with situs inversus (cited by Torgersen, 1947).

In the mass roentgenography series the anomaly was once found in a second cousin. As it is no unreasonable assumption that the 161 sibships correspond to about 10,000 second cousins, 1 case may depend on chance. It cannot be excluded, however, that the anomaly depends on a recessive gene in this particular case. Cockayne (1938) reported 2 instances of situs inversus occurring in uncle and nephew. The same reasoning may be applied concerning these cases. However, it can hardly be excluded that the observations are due to selection, partly because the chance of detection is greater in a family in which the anomaly has already been detected in one member, partly because the risk of selection is great in a relatively small material collected from the vast population of England, Canada, South Africa and Australia.

The frequency of first cousin marriages in the total material was 3.1 per cent, of second cousin marriages 3.5 per cent and of marriages between remoter relatives 3.5 per cent. In families showing the lung-nose syndrome the frequency of first cousin marriages was 5 per cent  $\pm 3.3$ , of second cousin marriages again 5 per cent. In the families not showing this syndrome the frequency of first cousin marriages was 2.5 per cent  $\pm 1.5$ . Among the familial cases there was one in which the parents were second cousins. According to Dahlberg (1943), the frequency of first cousin marriages in cases of an anomaly caused by a single recessive gene and showing a frequency of 0.01 per cent is expected to be 3.5 per cent if the frequency of first cousin marriages in the population is 0.5 per cent. According to Dunn, Dahlberg has found the frequency of first cousin marriages in country districts in Sweden to be 0.45 per cent. Thus, the frequency of first cousin marriages is somewhat too low to fit with the supposition of a single recessive gene. If there is a multifactor mechanism, one should expect a relatively higher frequency of consanguineous marriages. The established frequency of consanguineous marriages in connection with the deviation from the mendelian ratio indicates the importance of homozygosity not of a particular recessive gene but of a complex of genes which may be conceived of as modifiers conditioning

the coming into existence of visceral inversion. These modifiers may be assumed to promote the development toward normal or toward abnormal asymmetry by influencing the reactive potency of the embryo. The manifestation of single genes or that of nongenic factors may be assumed to be conditioned by these modifiers and the environment. The deficiency of consanguineous marriages may be partly due to nonhereditary cases.

The most striking fact is the lack of first cousin marriages in the familial cases and the lack of secondary cases in the 5 families in which the parents are first cousins, no case of situs inversus being detected in 30 siblings. This is strong evidence against the supposition of a single recessive gene. According to Haldane, a deficiency of consanguineous marriages may be due to a recessive gene which has a lethal allelomorph. According to the foregoing discussion this assumption is improbable in this case. Cockayne (1938) expressed the opinion that the partial inversions, which rather often are lethal, probably depend on allelomorphs of the recessive gene that causes complete situs inversus. There is no evidence confirming this supposition. As will be dealt with in the last section, there is good evidence that the relationship between partial and complete inversion has other explanations.

The high frequency of the lung-nose syndrome in the familial cases and the low frequency of consanguineous marriages in the familial cases indicate that the association of situs inversus and this syndrome is not due to the fact that the chance of homozygosity concerning more than one pair of rare genes is greater in consanguineous marriages. Also this observation is in conformity with the supposition of the importance of homozygosity of modifiers, in this case of modifiers influencing the development of the lungs. In only 1 of the 12 cases from Opland County were the parents first cousins. This confirms the supposition that the high frequency of situs inversus in this county is only partly due to hereditary factors.

I did not find evidence of linkage of bronchiectasis and situs inversus (Torgersen, 1947). The data suggested that the syndrome was due to genic factors which in some cases cause situs inversus as an expression of a pleiotropic effect. This interpretation was based on the finding that the syndrome was more common in the siblings and parents in the cases in which the patient with situs inversus showed the symptoms than in the other cases. I also attached importance to the parallelism in the development of the frontal sinuses, which were small in cases of situs inversus associated with bronchiectasis and of average size in cases without the syndrome.

These observations and interpretations are confirmed in the greater material of the present study. The syndrome occurred probably in 40 families. In these families the frequency of situs inversus in the siblings was 37 per cent, against 0.16 per cent in the siblings in the families in which the syndrome did not occur. The difference,  $3.54 \pm 1.57$ , is indicative that situs inversus is more frequent in the families showing the syndrome. In 3 instances in which the families of both parents showed the syndrome, 3 of the 12 siblings showed situs inversus. The consistency of the deviations are evidence that the chance that a child is going to show situs inversus increases with the homozygosity of the genic factors in this syndrome.

In the cases in which the syndrome has occurred in the families of the parents, the frequency of the symptoms in the offspring is 56 per cent, against 26 per cent in the cases in which the syndrome has not occurred in the families of the parents. The difference  $30 \pm 10.6$  may be regarded as significant, showing that

the frequency of the symptoms is higher in sibships deriving from a generation in which the syndrome has occurred

The behavior of the genic factors of this syndrome is similar to the behavior of genes showing incomplete dominance. The relative frequencies of consanguineous marriages recorded in a foregoing paragraph confirm this supposition. Bronchography was performed in 5 cases of situs inversus. In 3 cases bilateral bronchiectasis was revealed. In each of these 3 cases one of the parents was affected. In 2 cases localized bronchiectasis was found. In these cases the parents were not affected. These few cases show parallelism to the genetic evidence.

As seen in the introduction, there is striking conformity between the observations of the present study and the observations of Olsen at the Mayo Clinic concerning the nasal polyps. Nasal polyps occurred in 22 per cent of the cases of situs inversus with bronchiectasis, against 5 per cent of the other cases of situs inversus. The difference  $17 \pm 10$  indicates the same parallelism between the anomaly of the lungs and that of the frontal sinuses as was evident from the roentgen examination of the frontal sinuses. A frequency of nasal polyps of 5 per cent is hardly much above the frequency in the general population. However, the probability that this common condition belongs to the bronchiectasis and

TABLE 10—*Variations of the Spinal Column in Cases of Situs Inversus*

|  |                           |
|--|---------------------------|
| Number of spines examined in cases of situs inversus | 56                        |
| 6 lumbar vertebrae 10.7 per cent                     | } 23.2 per cent $\pm 5.8$ |
| Lumbosacral vertebra 12.5 per cent                   |                           |
| Spines examined in parents and siblings              | 61                        |
| 6 lumbar vertebrae or lumbosacral vertebra           | 5 per cent $\pm 2.9$      |
| Spines examined in normal situs                      | 500                       |
| 6 lumbar vertebrae 2.8 per cent                      | } 7.6 per cent $\pm 1.2$  |
| Lumbosacral vertebra 4.8 per cent                    |                           |

situs inversus is indicated by the alternating occurrence of bronchiectasis, nasal polyps and situs inversus in these families.

The observations are good evidence of genic factors in bronchiectasis and diseases of the frontal sinuses. The clinicians have to consider both genic and nongenetic factors. It is a common opinion that sinusitis may be the cause of bronchiectasis. The data set forth in the foregoing paragraphs indicate that both conditions may have a common genic basis.

The variations of the spine in the cases of situs inversus are seen in table 10.

The difference between the percentages of persons with situs inversus showing six lumbar vertebrae or lumbosacral vertebra and persons with normal situs showing these anomalies is  $15.6 \pm 7$ . The corresponding difference with respect to persons showing situs inversus and their siblings and parents is  $18.2 \pm 8.7$ . The variability of the spine is probably increased in patients with situs inversus. This does not hold in regard to the siblings or parents. The data offer no evidence that genes affecting the spine are a cause of situs inversus. Anomalies of the spine were detected accidentally in 3 per cent of the total material. There were 3 cases of hemispondylus, 1 case of severe scoliosis and 1 case of coccygeal defect and imperforate anus.

Observations of a particular family indicate that the increased variability of the spine may have a genic basis. A woman with six lumbar vertebrae and a hypoplastic kidney showed situs inversus and spina bifida occulta. Two of her brothers had the same combination of anomalies of the spine and situs inversus. One of them had a lumbosacral vertebra, and this was also the case with a third brother showing normal situs and spina bifida occulta. The frequency of

this anomaly in cases of situs inversus was 18 per cent, against 9.6 per cent in cases of normal situs, a difference far from statistically significant. It is improbable that this occurrence of familial increased variability of the spine, spina bifida occulta, situs inversus and hypoplastic kidney in one of the siblings is coincidental. Observations of man (Torgersen, 1948) and of the mutations of the tails of mice show the correlation between anomalies of the urogenital organs and anomalies of the spine. Imperforate anus may be classified in this group of anomalies. These findings in the spine are of interest in view of the experimental inversion produced by rotating pieces of the roof of the primitive gut. As will be seen from the following statements, there is good reason to assume that the primitive vessels play an important part in inversion. Sawin has demonstrated a correlation between the variations of the spine and those of the aortic branches and their symmetry in the rabbit. Kuhne (1931 and 1936) has shown the inheritance of the variations of the spine in man. Experimental evidence and observations of man suggest that variations in the development of the dorsal metameres play a fundamental role in the asymmetry of the viscera.

The morphology of the spine is of interest in the study of situs inversus not only because of the possibility that there is an inductive relationship between the dorsal region and the viscera but also because the heart and vessels take part in visceral asymmetry. The present material is selected as to the occurrence of heart defects. Such defects were noted in 10 cases in the total material and in 1 case in the mass roentgenography series. Cardiac defects occurred most probably twice in the 777 siblings. It is reasonable to refer briefly to the cases of heart defects even if the material is not representative because these cases illustrate some of the characteristic defects. In the literature there are a great many observations demonstrating that the correlation of situs inversus, a variety of heart defects and malformations of the body is rather high. There was 1 person who showed defect of the ventricular septum, pulmonary stenosis and complete situs inversus. There were further a hemivertebra (third thoracic) and 13 ribs on the right side. Another had an abnormally symmetric liver, a gallbladder to the left of the ligamentum teres, multiple spleens, a defect of the ventricular septum and a patent foramen ovale. A third had a symmetric liver, coarctation of the aorta, a defective lower jaw, syndactyly and a defective first rib on the left. A fourth showed complete situs inversus, transposition of the large vessels, with the aorta "riding" over a defect of the ventricular septum, and stenosis of the isthmus aortae. A fifth showed symptoms and signs of the tetralogy of Fallot and isolated dextrocardia. A sixth also showed the tetralogy of Fallot and isolated inversion of the abdominal organs, these anomalies representing a mirror image of those of the fifth. As to the remaining ones, the diagnosis of the heart defect is doubtful. In all, 3 of the 158 persons with complete situs inversus without heart defect showed anomalies of the body and extremities, against 3 of the 10 persons in whom situs inversus was complicated with heart defect. Heart defect occurred in 5 of the 158 persons who had complete situs inversus, against 5 of the 10 who had partial inversion. These observations are in conformity with the great many previous observations showing that associated anomalies are more common in patients with heart defects than in patients with situs inversus and, further, with the observations showing that heart defects are more common in patients with partial inversion.

There is no convincing evidence of hereditary relationship of cardiac malformation and situs inversus in the present material, the incidence of congenital heart disease in the siblings being 0.26 per cent, certainly not more than in the population generally. In 1 instance a child with situs inversus and heart

defect was born after seven years of marriage and two previous abortions. A maternal great aunt, a son of another maternal great aunt and a sister of the paternal grandfather were said to have died of congenital heart disease at 11, 14 and 20 years of age. The observations in the present study are not evidence of genes causing localized anomalies of the body and situs inversus as an expression of a pleiotropic effect. In 1 case a brother and an uncle of the mother of the child with situs inversus had harelip and cleft palate. One of the siblings died from congenital heart defect when about 1 year of age. In another case a first cousin had harelip. In 1 case the mother showed a short fifth middle phalanx, as did a sister of the patient in the index case of the family just mentioned. This is not more than might be expected in cases of anomalies with a frequency of about 0.1 per cent and 1 per cent, respectively.

One of the persons with situs inversus had been operated on several times because of acute intestinal obstruction due to abnormal rotation of the mesentery. It is impossible to state how often anomalies of the abdominal cavity occur in clinical cases. Autopsies showed a relatively high correlation between such anomalies and situs inversus.

The stomach of the twin with inversion of the abdominal organs is of particular interest. The shape is rather peculiar, the pyloric part not ascending but taking a horizontal position. This observation indicates that the factors of asymmetry have an influence on the shape of the stomach.

Among the persons with situs inversus there were 78 females and 90 males. As the chance of detecting the anomaly has been somewhat greater in males, the sex ratio is probably about 1:1. Among the siblings there were 382 females and 367 males and 30 of unknown sex.

About 7 per cent of the persons with known handedness were left handed—probably not more than in the population generally.

#### COMMENT

There is no evidence that the hereditary mechanism of the asymmetries produced in *Drosophila* and snails has any bearing on the asymmetry of the viscera of man. There is no reason to assume that the recessive mutation observed in mice is the only cause of situs inversus in this animal. Probably the mutations which in a few years have been detected causing situs inversus in mice are even more numerous than the mutations and modifiers influencing the development of the tail. As will be seen from the following considerations there is good evidence that the gills and their derivatives, the heart and the lungs, are of particular importance in situs inversus. For this reason one has to be cautious in attaching weight to experimental analogies concerning causative factors. The relationship between the gills and the heart is different in man, fish and amphibia. The development of the lungs is fundamental in the asymmetry of the viscera and of the heart in man.

The observations indicate that there is a connection between situs inversus and twinning in a few cases, partly due to genic, partly to nongenic factors. There is good reason to assume that in some of the cases of discordant twinning this is due to a mirror image effect.

caused by the late division. However, it is hardly possible to decide whether this mirror image effect is due to genic factors in bilateral differentiation. There is good reason to assume that conceptions as regulation, organizers, embryonic fields and inductive relationships are valid also in regard to man. However, it is difficult to decide at present to what degree such relationships depend on genic factors—a single gene or a few genes playing the part of the evocator, modifiers determining the reactive potency of the embryo.

The observations do not indicate a single factor mechanism as a general cause of situs inversus. It is reasonable to assume that the genic factors of bronchiectasis represent a very few of the genes responsible for the asymmetry of the viscera. Even if it is improbable that particular genes affect particular organs, the foregoing data are evidence that the development of the organs, as in the instance of the lungs, depends on particular genes, which at the same time influence the development of the asymmetry of the viscera.

In the third case in the casuistics cited in which a defective lower jaw was associated with an abnormality of symmetry and of the heart, the part played by the genes is obscure. The abnormality of the lower jaw and the asymmetry of the viscera in that case are not coincidental. Gruber found situs inversus in 6 of 82 cases of defective development of the lower jaw. The cases often showed multiple malformations of the heart and the extremities. Mohr reported a similar case in which the parents were first cousins.

Pernkopf (1926), from a morphologic point of view, concluded that the development of the viscera and the asymmetry of the organs were due to innate growth tendencies of the particular organs. That conclusion is supported by this study of the genetics of visceral inversion. Genes affecting the lungs may cause situs inversus in the same way as those affecting the lower jaw and the spine. The complex of genes affecting the organs and their asymmetry may be conceived of as modifiers. The present study is an approach to a breaking up of this complex of genes which are responsible for the asymmetry of the viscera.

Spitzer came to the conclusion that situs inversus depends on the structure of the cytoplasm, an opinion which is rather common among biologists (W. Ludwig, Harrison). The observations in man are in conformity with the assumption that the reactive potency of the embryo is one main factor in asymmetry reversal. The single gene or the genes which upset the balance play the role of the evocator. The observations indicate that the reactive potency depends on genic factors. As to the role of cytoplasm, they do not permit any conclusions.

The similarity between the development of sex and symmetry has been dealt with by W. Ludwig. He discussed the effect of the genic influence with respect to symmetry in terms similar to those used

by Goldschmidt in theorizing about the development of sex, the factors Right and Left taking the place of F and M

The not infrequent combination of localized anomalies such as harelip and visceral inversion or transposition is evidence that in man there is a correlation similar to that found by Komai in salmon. There is no evidence that localized anomalies caused by a relatively simple hereditary mechanism are combined with these malformations due to a pleiotropic effect of these genes. They may be considered as phenocopies, caused mainly by nongenic factors. The identical results of genic and nongenic factors demonstrate the fundamental role of the competence of the embryo. Such combinations are seen also in double monsters, and it is probable that the case of discordant situs inversus with mirror image harelip belongs to this group.

The data indicate that the factors modifying toward normal asymmetry are decisive in the development not only of the topography of the organs but also of the structure and of normal and pathologic function. This assumption was reasonable concerning the lungs and, as will be seen from the following considerations, a similar chain of causes is probable in regard to the development of the heart and of the abdominal organs.

It is of interest that the observations lead to the same conclusions which Landauer (1948) reported regarding the asymmetric expression of the genes in polydactyly of fowl. This study shows that the asymmetries of the body may have a genic basis. Sinistral expression of these genes gives response to selective breeding in the same way as bronchiectasis and situs inversus in cases of hereditary bronchiectasis. Landauer assumed that the asymmetric expression depends on an influence exerted on growth differentials by multiple genic factors. Lateral growth asymmetries may be supposed to be influenced by modifiers. This concept of modifying genes reveals the integrative interaction between the mutant gene and specific developmental potentialities of the embryonic field in which the gene takes effect. As will be seen, this reasoning and the reasoning concerning the observations in the present paper are in conformity.

The sporadic occurrence of situs inversus in connection with lack of signs of phenotypical expressions explaining a deficiency of ratio compared with the expected mendelian ratio, the possibly high age of the mothers and the concordance in a pair of dizygotic twins may be regarded as evidence that in not a few cases situs inversus is due to nongenic factors. The correlation with the malformations of the heart in which nongenic factors are important does indicate that nongenic factors may be of importance also in situs inversus. There is no case on record of situs inversus being associated with virus infection of the mother. Virus infection has been reported to cause the syndrome of aberrant right subclavian artery, an anomaly of symmetry which accord-

ing to Brean and Neuhauser, has been seen in association with transposition

The development of the asymmetry of the viscera and of the heart cannot be considered as an isolated process, the growth of the body taking place simultaneously. As far as I can see neglect to take this fact into consideration is the weakness of the theories of asymmetry reversal and transposition of the large vessels.

The influence of the growth differentials of the spine, lung buds and branchial arches indicates that the common mechanism of many cases of situs inversus is abnormal local growth rates. The vessels differentiate in situ, and in case of abnormal growth, in abnormal places. The joining of the vessels with the heart depends on their plasticity, which, according to the variability of the vessels, is great. In early stages a rearranging of the material during growth, in other words regulation or regeneration, may play a part tending to compensate for the abnormal growth rate. This may partly be the reason why so few of the persons with situs inversus show associated anomalies. These are more common in persons with heart defects, probably because the disturbances in the latter take place during a longer period. The abnormal growth concerns in most cases transitory structures, such as the gill arches, a fact explaining why the associated abnormalities tend to be transitory and why the anomalies of the heart so often have the character of atavistic phenomena.

The data given in foregoing pages indicate that abnormal symmetry or asymmetry of the vessels of the gill arches and of the aortic branches may produce either complete or partial inversion, particularly in the heart. Abnormal growth rates of the lung buds may cause abnormal position of the pulmonary vessels. The result will mostly be complete situs inversus, owing to the fact that the lung buds are related both to the foregut and to the heart. The other forms of partial inversion may be due partly to disturbances of growth rates of other parts of the body, partly to anomalies of the arteries of the branchial arches. The latter may extend their influences far caudally because of the caudal migration of the heart. Thus Pernkopf (1926) found symmetric pulmonary lobes in a case of isolated inversion in the abdominal cavity. A disturbance of the asymmetry of the organs of the septum transversum may cause inversion and anomalies of the heart from the caudal end as in the case showing inversion of the gallbladder and heart defect. The organs of the septum transversum and the derivatives of the midgut have a rather independent position in asymmetry. The diaphragm is often the border between a caudal and a cranial inversion. The stomach, duodenum, liver, gallbladder and large bowel may show isolated inversion and abnormal symmetry both in topography and in structure. The abnormal spleen in the person



with situs inversus and heart defect is probably due to abnormal asymmetry of the dorsal mesentery Toldt (1889) attached importance to the symmetry of the dorsal mesentery in the development of the spleen

E Ludwig and Pernkopf (1926) took it for granted that bilateral organs, such as the lungs, have no influence on the situs viscerum The evidence of the present study indicates the opposite, that the situs of the viscera depend on bilateral growth differentials of the viscera and the body and corresponding changes in the position of the vessels Probably for this reason the genic mechanism of bilateral expression of the genes and that of asymmetry reversal of the viscera show striking similarities

The conception that the embryonic vascular bed is an important factor of heart defects is not new Taussig (1947), referring to Streeter came to the same conclusion According to her experience, the anomaly most commonly associated with heart defects is malformation of the spine, a finding which conforms with the observations concerning situs inversus

The correlation of growth of the body and defect of the heart is evident in cases of mongolism and of arachnodactyly According to Abbot, transposition has been recorded in cases of mongolism That there is an influence on the asymmetry of the viscera in these cases is evident from the frequent symmetries of the lobes of the lungs

I have not found any data showing that asymmetries of the body are commonly associated with heart defects According to Brown, supernumerary nipple is one of the most common anomalies in cases of heart defects This observation is of interest in view of the observation of Landauer (1939) that there is a correlation between an abnormality of symmetry, such as left-handedness, and supernumerary nipple

Huxley, discussing relative growth, defines as instances of accretionary growth cases in which the material which is added is not alive Such growth processes may result in spirals In a study of the comparative anatomy of the stomach (Torgeisen, 1942) I came to the conclusion that accretionary growth in this sense represents a particular case of a more general morphogenetic process concerning cases in which layers have different rates of growth The layer showing the slowest rate has an influence on the form similar to that of the dead material

The transposition of the large vessels and the possibility that this anomaly is related to visceral inversion have called on the interest of some of the most brilliant authors in different fields of biology and medicine (Harris and Farber)

To proceed from the cranial part, abnormal development of the mandibular arch and of the embedded vessels may cause complete asymmetry reversal and cardiac defect, a right subclavian artery from the left side

of the aorta may be associated with transposition. In the tetralogy of Fallot, in which transposition is one of the abnormalities and which is often combined with complete or partial inversion, particularly of the heart, a right-sided aortic arch, a mirror image of the normal, is found in 25 per cent of the cases.

Genic and nongenic factors may be supposed to affect the growth of the metameres or of the branchial arches, the most rapidly growing parts in different stages of development being most sensitive to growth-inhibiting influences. This causes abnormal asymmetry of the vessels in the corresponding regions. The latter condition puts a stress on the developing heart tube resulting in abnormal asymmetry of the bulbus and the common arterial trunk. This abnormal asymmetry affects the torsion of the truncus-bulbus septum and the bulboventricular loop in a different way. The truncus and the cranial part of the bulbus develop in a relatively fixed mesodermal sheet. Both Spitzer and Pernkopf and Wirtinger attach importance to the fixation of both ends of the cardiac tube. The bulboventricular loop is loosely fixed. The inner layer is growing faster than the outer layer, expanding the latter. In the relatively fixed truncus the torsion can take place only between the layers, manifesting itself in the truncal septum and the bulbar ridge. The process corresponds to that which, according to Jacobshagen (1931, 1934), takes place in the development of the spiral valve in the fixed mesodermal sheet in the spiral intestine of fish. The part of the bulboventricular loop that is not fixed undergoes a relatively free topographic torsion in a way which provides for a meeting of the septums. The explanation of the precision of these processes, according to this theory, is that they are different manifestations of the same causes. Abnormal asymmetric or symmetric influences may produce the different types of transposition with or without inversion.

This theory is similar to that of Pernkopf and Wirtinger. According to these authors who based their reasoning on embryologic evidence, a partial inversion is the cause of transposition. Spitzer and Pernkopf agreed that partial inversion may occur in different sections of the heart.

The bulboventricular loop does not undergo an entirely free torsion. In relation to the small intestine the heart tube is relatively fixed in the same way as the stomach. This is expressed in the muscular structure of both heart and stomach. Taussig (1926) and Pernkopf (1926) found that the superficial layer was not reversed, in contrast to the middle layer, which showed a mirror image asymmetry. The structure of the musculature and the formation of the vortex are influenced by the asymmetry reversal. Thus the torsion which depends on the strain put on the heart tube by the embryonic vessels produces at the same time the orientation of the septums and the organization of

the musculature which are fundamental in hemodynamics. In later stages the blood current may be assumed to be essential in the normal and in the abnormal development of the heart. These data are good evidence that the modifiers concerned with asymmetry are fundamental not only in the topography of the viscera but in the structure and in the normal and the pathologic function as well. This is confirmed by the observations of the anatomy of the stomach, which will be dealt with in subsequent paragraphs.

The torsion of the mesentery is an example of free torsion without any influence on the structure of the organ. There is no fundamental difference between the torsion of the mesentery and those of the bulboventricular loop, the stomach, the bulbus-truncus septum and the spiral valve. This is indicated in the fact that abnormal torsion of the mesentery and abnormal symmetries in the abdominal cavity are commonly associated with situs inversus and cardiac defects. These observations and the high correlation of the most different anomalies of the heart and situs inversus are good evidence that in cardiac defects the fundamental process is a disturbance of symmetry.

There are a few cases on record indicating a hereditary relationship between situs inversus and cardiac defect, among them the cases reported by Pernkopf (1937) and Roesler. The latter reported a case in which transposition occurred with partial inversion of the heart in one brother and clinical congenital heart disease in another. The parents were first cousins. The observations of familial occurrence of heart defects are convincing evidence of the influence of genic factors. The high correlation of heart defect and visceral inversion is evidence that the modifiers concerned with asymmetry are of importance in the mechanism of inheritance of heart defects. This does not exclude the influence of single genes.

The studies of Pernkopf (1926, 1937) and data in the literature show that the viscera in situs inversus may have abnormal shape and structure. This may have some interest, owing to the predisposition toward ileus in abnormalities of the mesentery.

The peculiar shape of the stomach of the twin showing isolated abdominal inversion is of particular interest. Pernkopf (1926) found the same shape of the stomach in 2 cases of isolated inversion of this organ. What surprised him was the observation that there was no pyloric sphincter in either of these two stomachs, one of them not even showing a pyloric sulcus. The pyloric part of the stomach looked like a part of the intestine. In a third of these extremely rare cases he found a strong pyloric musculature, looking like a case of pyloric spasm. This case showed the unique combination of an inverted stomach and a normal, not inverted, duodenum. For this reason the transition from the pyloric part of the stomach to the duodenum showed

a sharp angle. In the 2 first cases and possibly in the case in the present study the malformation was in a way the counterpoint to the hypertrophy observed in infantile pyloric stenosis and has, as far as I know, not been observed in the normal situs. In the study of the stomach I found that the growth differentials of the curvatures and the relative fixation of the stomach toward the transition to the midgut may be supposed to be of particular importance to the development of the pyloric musculature. The modifiers concerned with asymmetry are of importance in this fixation of the caudal part of the stomach, in the same way as they are essential in the fixation of the mesentery generally. The case in which the stomach was inverted and the duodenum not inverted confirms these suppositions, the pyloric part being abnormally fixed by the peculiar position of the duodenum.

It is evident that infantile pyloric stenosis may have a genic basis. The attempts to demonstrate a "single factor" mechanism have not been convincing. There is reason to assume that the modifiers which are fundamental in asymmetry are fundamental also in this disease. These children show in a way an exaggerated development of a gastric muscular structure which depends on the asymmetry of the organ. In contrast with children presenting congenital anomalies, they have been found in most cases to be taller than the average when examined as "grown ups" (Salmi). Like the patients with ulcer, they belong mostly to the slender type. Concerning the alimentary diseases observed in the families of children with pyloric stenosis, Cockayne and Penrose have supposed that some of these diseases may be manifestations of the gene in the heterozygous form. It might be added that the conformity noted as to sex ratio and body build in cases of ulcer of the stomach and duodenum and cases of infantile pyloric stenosis indicates a common genic basis also concerning the modifiers.

Complete situs inversus was probably about equally frequent in the sexes. In cases of persistent truncus arteriosus and cases of transposition taken together there are, according to White and Monckeberg, 72 per cent boys, in cases of pyloric stenosis there are 80 per cent boys. There is reason to assume a sex difference with regard to the modifiers of the development of asymmetry and the related modifiers of the development of the particular organs. Conditions indicating a high degree of asymmetry or of abnormal symmetry are more common in males. According to Cummins and Midlo, a decrease of the bilateral asymmetry of the fingerprints is seen in left-handed males, and increase in left-handed females, the net effect being a leveling of the sex distribution of bilateral asymmetry, females being more symmetric generally. Left handedness is more common in boys. The correlations between this anomaly and supernumerary nipples and between the latter and heart defects are evidence of a relationship between sex, symmetry and

heart defects, a strong gynec component tending to produce abnormal symmetry in males. The similarity as to the influence of sex in different groups of asymmetries is good evidence of a similarity as to the genic mechanism. Sex influences the expression of the genes in much the same way as laterality does, both factors being of importance also in the expression of genic factors having relationship to the asymmetry of the viscera. Perhaps the most reasonable interpretation may be that the autosomes, which are supposed to have a masculine effect, also have a prominent effect on growth and bilateral growth differentials. These effects may be supposed to be modified by the X chromosomes, which may be assumed to have an opposite effect concerning all three features.

Dunn and Landauer (1934, 1936) suggested that the different expression of the tail mutations in fowl is due to modifiers that have been accumulated for the sake of developmental safety. This conception is reasonable also in regard to the modifiers concerned in visceral asymmetry. The essential point of Spitzer's theory of transposition is that the septation of the heart and the development of the lungs and the pulmonary artery were necessary adaptations to terrestrial life and that the torsion of the heart tube has played a part in this development. Keith (1913) attached importance to the regression of the bulbus in accounting for pulmonary stenosis. The regression of the bulbus depends on the asymmetric growth of the bulboventricular loop. According to Saphir and Lev, the regression of the bulbus is important also in transposition. Keith stressed the phylogenetic correlation of the bulbus and the gills in an anatomic comparison parallel to the observations and interpretations of the present study.

Spitzer considered the heart in transposition as a reptilian heart in man. He expressed the opinion that transposition has phylogenetic causes, visceral inversion ontogenetic causes. Transposition is an expression of a disturbance of what he called a "metastable equilibrium." Has this metaphor from physics any biologic sense? The reappearance of a reptilian heart in man, even in details, may indicate that the mutations and selection concerning the heart in phylogeny have represented a continuous process giving discontinuous results. At a particular step in the accumulation of modifiers fundamental changes may have occurred in the reactive potency of the embryo. The genic complex in the development of the heart may be in a metastable equilibrium, mutations making the heart recoil on one of the discontinuous and decisive steps in its evolution. The observations indicate that the genic complex in the asymmetry of the viscera has been important in these processes. They do not confirm Spitzer's postulation that transposition and inversion are quite different phenomena. The objection to Spitzer's theory that a reptilian heart is not seen in human ontogeny may be regarded as irrelevant. In the evolution of the heart bilateral growth differentials

and alternative variability have played a great part. The alternative that is not realized leaves no trace is normal ontogeny. It reveals its presence as a possibility in the abnormal development of the heart.

#### SUMMARY

This study is based on 168 cases of situs inversus viscerum observed in Norway. They were partly detected in a mass roentgenography series comprising 1,000,000 persons, one third of the population. It is regarded as an advantage that the cases were collected from a small and geographically limited population. From the point of view of genetics all the cases must be regarded as selected. From the point of view of pathology they are partly selected, about one half of them having been reported by physicians. In all about 66 per cent of the persons with situs inversus in the country have been detected. The possibility that a very few involved siblings have not been detected is compensated for by the inclusion of two familial cases recorded by other authors. The plan of the study has been to make an approach by means of this abnormality of symmetry to the part played by the factors of visceral asymmetry in the development and the pathologic changes of the viscera, particularly the heart, the lungs and the stomach.

In Norway the frequency of situs inversus is probably somewhat above 0.01 per cent. There is evidence of geographic variations which parallel the variations of the frequency of twinning. The causes of these variations are discussed.

The frequency of situs inversus is not much higher in monozygotic twins than in the population. In some of the few families in which many twins occurred, the patient with situs inversus was a twin, a fact indicating a relationship between twinning and this anomaly in a few cases.

Situs inversus is probably rare in dizygotic twins. Among the cases in the present study is one in which the anomaly was found in both of a pair of dizygotic twins. The theoretic aspect of situs inversus occurring in dizygotic twins is discussed.

The age of the mothers is probably high in the cases complicated with malformations of the heart or with bronchiectasis.

The ratio of affected to normal persons does not accord with the supposition that a single recessive gene is generally the cause of situs inversus. In the present material there is no evidence of an increased prenatal selection or of unknown phenotypes which may explain why the actual ratio falls short of the expected ratio in recessive inheritance.

The low frequency of marriages of first cousins in the familial cases and the lack of involved siblings in cases in which marriages of first cousins were noted are difficult to match with the supposition of a single recessive gene.

Situs inversus is more equally distributed between the sexes than heart defects and transposition of the great vessels. The genic basis of the correlation between asymmetry, general growth and sexual differentiation in different groups of asymmetries in man is discussed.

Anomalies of the spine are increased in persons with situs inversus, as well as defective development of the lower jaw. This may indicate inductive relationships or an influence of abnormal growth rates. These findings are good evidence that the growth rates of the gills and the metameres and accordingly the position of the embedded vessels are of importance in many cases of asymmetry reversal and heart defects.

The relationship between situs inversus and heart malformation may be regarded as a clue to an understanding of the developmental processes and causes of congenital heart disease. On this basis a theory is proposed concerning the transposition of the large vessels, including the main points in the previous theories of Pernkopf, Wirtinger, Spitzer and Keith.

Bronchiectasis occurred in about 25 per cent of the cases and in 4 of the 5 familial cases. This frequency of bronchiectasis and nasal polyps is discussed, together with the difficulties of their diagnosis. Bronchiectasis and nasal polyps may occur as different manifestations of a common genic basis. The frequent coincidence of nasal polyps and bronchiectasis and the fact that the frontal sinuses are small in cases of situs inversus complicated with bronchiectasis and not in the other cases may be regarded as good evidence of a common genic basis of bronchiectasis and nasal polyps. The syndrome behaves as a dominant with varying expression and chance of inversion in heterozygotes and homozygotes. This combination may be a clue to the understanding of the relationship between genic factors in the morphology and the asymmetry of particular organs and the asymmetry of the viscera. Evidence from morphology and genetics indicates that the asymmetry of the viscera is due to a complex of genes which may be broken up into components of particular importance in particular organs. The complete asymmetry depends on the integrative action of this complex of genes. The role of these modifiers in normal asymmetry in the morphologic and pathologic aspects of the viscera is discussed with particular regard to the lungs, the heart and the stomach. A theory is proposed concerning the influence of these modifiers in infantile pyloric stenosis. The genic aspects of the phylogenetic theory of Spitzer concerning transposition are discussed.

#### ADDENDUM

Since the manuscript was submitted for publication the material has increased substantially. It now includes 185 families and 195 cases of situs inversus. About 1,500,000 persons have been examined.

by mass roentgenography. In the following paragraphs are recorded some data of interest in the present study.

Twin birth occurred in 1.7 per cent of 1,082 births in the sibships. Of 57 families, one of the grandparents was a twin in 3, and one of the parents in 2—corresponding to a frequency of 1.3 and 1.7 per cent respectively. In a sibship of 9 situs inversus occurred in 2 siblings. The father was a twin. Situs inversus has not been detected among the siblings in the cases recorded. Two more familial cases have been detected. Besides the family in which the father was a twin, a sibship was detected in which 3 siblings showed situs inversus and 1 the normal situs. The parents were second cousins. The lung-nose syndrome occurred in both these families, all the 5 persons with situs inversus showing symptoms of bronchiectasis, 2 of them nasal polyps as well. The mean age of the mothers in 191 cases is 31.4. The difference from the age of mothers at birth generally is  $0.9 \pm 0.43$ . The numbers of affected and normal siblings, the index cases excluded, are 10 and 897 respectively. The frequency of first cousin marriages is 3.2 per cent, of second cousin marriages 7.7 per cent. In families showing the lung-nose syndrome the frequency of first cousin marriages is 6 per cent and of second cousin marriages 16 per cent. In the other families the corresponding frequencies are 2.2 per cent and 3.8 per cent. The difference between the groups concerning consanguineous marriages is  $16 \pm 7.2$ . No situs inversus has been detected in 39 siblings in the 6 families in which the parents were first cousins. In 45 families showing the lung-nose syndrome the frequency of situs inversus in the siblings was 5 per cent, against 0.16 per cent in the siblings in the other families.

The supplementary data confirm the interpretations in the preceding pages. The data are evidence of an interaction between genes affecting the particular organ or regions connected with it and a genic complex which gives response to selective breeding and influences the growth differentials determining the asymmetry of the viscera.

#### BIBLIOGRAPHY

- Abbott, M. E., in *Nelson Loose-Leaf Medicine*, New York, Thos. Nelson & Sons, 1937, vol. 4, p. 207.
- Adams, R., and Churchill, E. J. *Thoracic Surg.* **7**: 206, 1937.
- Aschoff, L. *Arch. f. Entwicklungsmech. u. Organ.* **116**: 267, 1929.
- Brean, H. P., and Neuhauser, E. B. D. *Am. J. Roentgenol.* **58**: 708, 1947.
- Brown, J. W. *Congenital Heart Disease*, London, John Bale, Sons & Curnow, Ltd., 1939.
- Cockayne, E. A. *Quart. J. Med.* **31**: 479, 1938; *Biometrika* **31**: 287, 1939.
- and Penrose, L. S. *Ohio J. Sc.* **43**: 1, 1943.
- Cummins, H., and Midlo, C. *Finger Prints, Palms and Soles. An Introduction to Dermatoglyphics*, Philadelphia, The Blakiston Company, 1943.



- Dahlberg, C Twin Births and Twins from a Hereditary Point of View, Uppsala, Kungl Universitet, 1926, Acta med Scandinav, 1943, supp 148, Nord med **37** 24, 1948
- Diehl, K, in Abel, W, and others Handbuch der Erbbiologie des Menschen, Berlin, Julius Springer, 1940, vol 4, p 96
- Doolittle, W F Boston M J **157** 662, 1907
- Dubreuil-Chambardel, L Presse med **35** 1157, 1927
- Dunn, L C Proc Nat Acad Sc **33** 359, 1947
- and Landauer, W J Genetics **29** 217, 1934, **33** 401, 1936
- Feldman, W M Proc Roy Soc Med **38** 735, 1935
- Frolich, T Norsk mag f lægevidensk **81** 119, 1920
- Gansslen, M, Lamprecht, K, and Werner, M, in Abel, W, and others Handbuch der Erbbiologie des Menschen, Berlin, Julius Springer, 1940, vol 4, p 198
- Gruber, G B Stud z Pathol d Entwcklmg **2** 405, 1920
- Gutzeit, K, and Lehmann, W, in Abel, W, and others Handbuch der Erbbiologie des Menschen, Berlin, Julius Springer, 1940, vol 4, p 581
- Haldane, J B S Ann Eugenics **8** 263, 1938
- Harris, J, and Farber, S Arch Path **28** 427, 1939
- Harrison, R C Tr Connecticut Acad Arts & Sc **36** 277, 1945
- Helweg-Larsen, H F Ann Eugenics **14** 1, 1947
- Huxley, J S Problems of Relative Growth, New York, Lincoln MacVeagh, The Dial Press, 1932
- Jacobshagen, E Morphol Jahrb **67** 677, 1931, **73** 392, 1934
- Joyce, J C Brit M J **2** 548, 1945
- Kartagener, M Ergebn d inn Med u Kinderh **49** 378, 1935
- Kean, B H J Hered **33** 217, 1942
- Keith, A Lancet **2** 359, 1909, Human Embryology and Morphology, London, Edward Arnold, 1913
- Komai, T Mem Coll Sc Kyoto Imp Univ s B 1938, vol 15
- Kuhne, K Ztschr f Morphol u Anthropol **30** 1, 1931
- Landauer, W Human Biol **11** 447, 1939, J Genetics **30** 403, 1945, **33** 133, 1948
- Lopez, Areal, L Rev clin españ **14** 378, 1944
- Ludwig, E Morphol Jahrb **55** 270, 1925
- Ludwig, W Rechts-Links-Problem im Tierreich und beim Menschen, Berlin, Julius Springer, 1932
- Mattison, K Ztschr f menschl Vererb - u Konstitutionslehre **17** 325, 1933
- Mohr, O L Heredity and Disease, Toronto, George J McLeod, Ltd, 1936
- Monckeberg, J G, in Henke, F, and Lubarsch, O Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1927, vol 2
- Murphy, D P Congenital Malformations, Philadelphia, University of Pennsylvania Press, 1940
- Natvig, H Nord med **1** 681, 1939
- Ochsenius, K Monatsschr f Kinderh **19** 27, 1921
- Olsen, A M, in Hewitt, R M, and others Collected Papers of the Mayo Clinic and Mayo Foundation, Philadelphia, W B Saunders Company, 1942, vol 34, p 764
- Penrose, L S J Genetics **25** 407, 1932, The Influence of Heredity on Disease, London, H K Lewis & Co, Ltd, 1934, Ann Eugenics **13** 73, 1947

- Pernkopf, E Ztschr f Anat u Entwicklsgesch **79** 577, 1926, **87** 661, 1928,  
Ztschr f menschl Vererb - u Konstitutionslehre **20** 607, 1937
- and Wirtinger, W Virchows Arch f path Anat **295** 143, 1935
- Reinhardt Deutsche mil-arztl Ztschr **41** 932, 1919
- Roberts, J A F An Introduction to Medical Genetics, London, Oxford Uni-  
versity Press, 1940
- Roesler, H Arch f inn Med **19** 518, 1930
- Salmi, T Acta pædiat **28** 270, 1941
- Saphir, O, and Lev, M Am Heart J **21** 31, 1941
- Sawin, P B Anat Rec **100** 76, 1948
- Spemann, H, and Falkenberg, H Arch f Entwicklgsmechn d Organ **45** 371,  
1919
- Spitzer, A Virchows Arch f path Anat **271** 226, 1929
- Streeter, cited by Taussig (1947)
- Taussig, H B Bull Johns Hopkins Hosp **39** 199, 1926, Congenital Malforma-  
tions of the Heart, New York, The Commonwealth Fund, 1947
- Tihen, A, Charles, D R, and Sippel, T O J Hered **39** 29, 1948
- Toldt, C, cited by Pernkopf (1926)
- Torgersen, J Acta radiol, 1942, supp 95, Acta med Scandinav **126** 319,  
1946, Acta radiol **28** 17, 1947, **29** 311, 1948, J Hered **39** 293, 1948, to be  
published
- Weinberg, W, cited by Luxenburger, in Abel, W, and others Handbuch der  
Erbbiologie des Menschen, Berlin, Julius Springer, 1940, vol 2 p 213
- White, P D Heart Disease, New York, The Macmillan Company, 1945
- Wilson, J G, and Warkany, J Anat Rec **100** 92, 1948
- Wright, S, cited by Landauer (1948)

## EFFECT OF TRIPELENNAMINE HYDROCHLORIDE ON BURN SHOCK

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SINCE the work of Dale and Laidlaw,<sup>1</sup> it has been recognized that histamine or a histamine-like substance may be responsible in part for the development of shock. The evidence in support of this theory has, however, been conflicting. Moon<sup>2</sup> and Harkins<sup>3</sup> have recently presented reviews on this subject. The discovery of drugs, such as pyramisamine (neoantergan,<sup>®</sup> [N,N-dimethyl-N'-(p-methoxybenzyl)-N'-(alpha-pyridyl) ethylenediamine]), diphenhydramine (benadryl<sup>®</sup> [beta dimethylaminoethyl benzhydryl ether]), tripeleNNamine (pyribenzamine<sup>®</sup> [N,N-dimethyl-N'-Benzyl-N'-(alpha-pyridyl) ethylenediamine]) and others, which have the properties of histamine antagonists, suggested a new means of investigating the role played by histamine in shock. Friedlander, Feinberg and Feinberg,<sup>4</sup> and Sherrod, Loew and Schloemer<sup>5</sup> have shown that tripeleNNamine will prevent shock from developing after intravenous injection of histamine in guinea pigs and dogs. However, Jourdan and Chatonnet<sup>6</sup> and Ingraham and Wiggers<sup>7</sup> have reported that pyramisamine and diphenhydramine do not alter the course of either traumatic or hemorrhagic shock. The purpose of the experiments to be described was to extend the work of these authors and to study the effects of tripeleNNamine in burn shock.

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1 Dale, H. H. *Lancet* **1** 1179, 1929. Dale, H. H., and Laidlaw, P. P. *J. Physiol.* **41** 318, 1910, **52** 355, 1919.

2 Moon, V. H. *Shock: Its Dynamics, Occurrence and Management*, Philadelphia, Lea & Febiger, 1942.

3 Harkins, H. N. *The Treatment of Burns*, Springfield, Ill., Charles C. Thomas, Publisher, 1942.

4 Friedlander, S., Feinberg, S., and Feinberg, A. R. *J. Lab. & Clin. Med.* **32** 47, 1947.

5 Sherrod, T. R., Loew, E. R., and Schloemer, H. F. *J. Pharmacol. & Exper. Therap.* **89** 247, 1947.

6 Jourdan, F., and Chatonnet, J. *Compt. rend. Soc. de biol.* **137** 559, 1943.

7 Ingraham, R. C., and Wiggers, H. C. *Federation Proc.* **5** 50, 1947.

## METHODS AND MATERIALS

Fourteen female albino rabbits were used in this experiment. These animals had previously been used for the Friedman test but were in good health, and the incisions were well healed. Seven animals were used as controls and 7 received tripeleNNamine<sup>8</sup>. The treated group were given 4 mg of tripeleNNamine hydrochloride per kilogram of body weight. The drug was dissolved in isotonic solution of sodium chloride and injected into the marginal vein of an ear. These animals were given 1 mg of the drug per kilogram every two hours until the conclusion of the experiment. Ether anesthesia was used to maintain unconsciousness during the period of burning and for a few minutes thereafter. Animals 1 and 8 were clipped, but the fur of the remaining animals was soaked thoroughly with water to insure even heat conduction before burning. A control blood sample

*Comparison of the Hematocrit Readings Made for Rabbits Treated with 4 Mg of TripeleNNamine Hydrochloride per Kilogram of Body Weight and Untreated Rabbits (Controls), All of Which Were Immersed in Water at 80 C for Ten Seconds*

| Animal<br>Treated | Hours After Burn |     |     |     |     |     |     |     |     |    |     |    |    | Cause of<br>Death | Survival,<br>Hr |
|-------------------|------------------|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|----|----|-------------------|-----------------|
|                   | 0                | 2   | 4   | 6   | 8   | 10  | 12  | 14  | 16  | 18 | 20  | 22 | 24 |                   |                 |
| 1                 | 41               | 48* | 45* |     | 28  |     |     |     |     |    |     |    |    | Shock             | 8               |
| 2                 | 39               | 39  | 36  |     |     |     |     |     |     |    |     |    |    | TripeleNNamine?   | 5               |
| 3                 | 49               | 50  |     |     |     |     |     |     |     |    |     |    |    | TripeleNNamine?   | 2               |
| 4                 | 42               | 47* | 47* |     |     |     |     |     |     |    |     |    |    | Shock             | 4               |
| 5                 | 41               | 41  | 54* |     | 50* |     | 42  |     | 46  |    | 41  |    |    | Convulsions       | 25              |
| 6                 | 35               | 45* |     | 42* |     |     |     |     |     |    |     |    |    | Hemopericardium   | 7               |
| 7                 | 38               | 57* | 49* | 51* |     | 50* |     |     |     |    |     |    |    | Shock             | 12              |
| Untreated         |                  |     |     |     |     |     |     |     |     |    |     |    |    |                   |                 |
| 8                 | 43               | 41  | 51* |     | 47* |     | 48* |     | 47* |    | 46* |    | 40 | Put to death      | 27              |
| 9                 | 42               | 53* | 51* |     | 55* | 53* |     |     |     |    |     |    |    | Shock             | 10              |
| 10                | 42               | 34  | 51* |     | 50* |     | 47* |     | 47* |    | 46* |    | 44 | Hemopericardium   | 27              |
| 11                | 43               | 56* | 56* | 56* |     | 48* |     | 45* | 43  |    |     |    | 38 | Hemopericardium   | 23              |
| 12                | 32               | 32  | 29  |     | 39* |     | 37* |     | 32  |    | 30  |    |    | ?                 | 40              |
| 13                | 44               | 16? | 54* |     | 56* |     | 52* |     | 48* |    | 49* |    | 48 | Put to death      | 52              |
| 14                | 40               | 43* | 44* |     | 45* |     | 40  | 38  |     |    | 38  |    | 36 | Pneumonia         | 36              |

\* The animal was in shock

was drawn from each rabbit by cardiac puncture, and the animals were then immersed to the xiphoid in water at 80 C for ten seconds. The rabbits were placed in cages and fed a commercial rabbit chow and as much water as they would drink.

Hematocrit determinations were made for all animals at two to four hour intervals, and for 8 animals erythrocyte counts were also made. Blood samples were drawn by cardiac puncture. Survival time was noted, and complete autopsy was done immediately after death.

## RESULTS

Twelve of the 14 animals in this series underwent shock, which was recognized by an increase in the hematocrit value and in the red blood

<sup>8</sup> Pyribenzamine hydrochloride,<sup>®</sup> supplied by Ciba Pharmaceutical Products, Inc., was used.

cell count, by pallor of the ear, coldness and lethargy. The pulse and respiratory rates were too variable to be used for analysis. Two of the animals in each series were burned for twenty seconds, but the results were not altered by this increase in length of burning. Animals dying of hemopericardium and those dying before the onset of shock were eliminated from the studies of longevity.

Of the 7 animals which received tripeleennamine, 5 showed evidences of shock. Two animals died within five hours of burning without revealing hemoconcentration and were therefore eliminated from the series. Four animals died in shock. One animal died of hemopericardium after recovering from shock. The average length of life in this group was ten hours, and all except 1 animal died within four to twelve hours.

In the control series all 7 animals presented evidences of shock, but 6 of them recovered from the shock before death. One animal died of hemopericardium while in shock. Two were killed after recovery to avoid infection of the burned areas, 1 died of hemopericardium, and 1 of pneumonia after recovering from shock. The average survival time for this group was thirty-three hours, with all but 1 living twenty-seven to fifty-two hours.

Of the 5 shocked animals in the treated series, 4 showed first signs of hemoconcentration at two hours and 1 at four hours. In the control series, hemoconcentration tended to occur later, with 3 showing first signs of increased cell volume at two hours, 3 at four hours and 1 at eight hours.

The highest hematocrit reading was 57 in the treated group and 56 in the control group, the average hematocrit peak being 50 in both groups. The peak hematocrit values tended to occur later in the control group. The average maximum increase of the control group was 23 per cent and that of the treated group 26 per cent. The range of percentage increase was 12.5 to 31 in the control group, and 12 to 50 in the treated group.

The erythrocyte counts checked with the hematocrit readings. The changes observed at autopsy in one series of animals were similar to those observed in the other. Shock was accompanied by pulmonary edema, hyperemia of the viscera and engorgement of the veins of the abdomen.

#### COMMENT

In 1939 Wense<sup>9</sup> induced burn shock in normal animals and in animals desensitized to histamine or animals previously given torantil<sup>®</sup> (desiccated kidney and extract of the mucous membrane of the small intestine of the hog, containing histaminase) and found no difference in

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<sup>9</sup> Wense, T. *Ztschr f Immunitätsforsch u exper Therap* 97 100, 1939

the survival time but thought the local reaction less in the treated animals than in the control animals. If histamine were the "burn toxin" producing the vascular collapse and shock after severe burns, it would seem that tripeleNNamine, if an effective antagonist of histamine, should alter the course of events after a standard burn. However, in these studies no decrease of severity, delay of onset or inhibition of progression of shock was noted. The only significant change was decreased viability of the animals given tripeleNNamine hydrochloride. The drug, therefore, seems to exert no effect on the mechanism of burn shock and acts only as an additional toxic factor in an already traumatized animal.

#### SUMMARY

Burn shock was induced in 12 of 14 rabbits. Seven of the animals received large doses of tripeleNNamine hydrochloride intravenously.

No decrease of severity, delay of onset or inhibition of progression of shock was noted in the treated series.

The treated series showed decreased viability.

# PRIMARY SYSTEMIC AMYLOID DISEASE

Report of a Case Emphasizing Cardiac Involvement

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**A**MYLOID is a peculiar member of the group of connective tissue hyalins, set apart from the others by certain identifying characteristics. These are its staining reactions,<sup>1a</sup> its appearance in persons who have passed through a long wasting illness<sup>1b</sup> and the fact that it involves principally the parenchymatous organs (liver, spleen and kidneys) and the adrenal glands.<sup>1c</sup> Lubarsch<sup>2</sup> is recognized as the first to point out cases in which a hyaline eosinophilic substance, possessed of certain of the morphologic properties of amyloid and conspicuously lacking others, makes its appearance in tissue. These cases are designated as instances of "primary systemic amyloidosis", they are recognized by criteria initially formulated by Lubarsch and employed by subsequent writers.<sup>3</sup> In what is deemed the order of their importance, these are (a) absence of such a predisposing factor as one of the chronic suppurations, (b) failure of the amyloid deposit to show the usual staining reactions, (c) no involvement of the organs commonly affected by secondary amyloidosis, (d) extensive amyloidosis of such sites as the heart, blood vessels, skin and skeletal muscle not commonly involved in secondary amyloidosis.<sup>3a</sup> In these cases the infiltrate resembles that seen in amyloidosis secondary to pulmonary tuberculosis, chronic osteomyelitis, infected tumors, leprosy, chronic nephritis and other diseases<sup>1a</sup> in its gross appearance and in its reaction to the routine histologic stains. Its response to the special stains for amyloid, i. e., dilute Lugol's solution and sulfuric acid, congo red and crystal violet,

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1 (a) Karsner, H. T. Human Pathology, Philadelphia, J. B. Lippincott Company, 1942. (b) MacCallum, W. G. A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1936. (c) Smith, L. W., and Gault, E. S. Essentials of Pathology, New York, D. Appleton-Century Company, Inc., 1942.

2 Lubarsch, O. Virchows Arch f path Anat 271 867, 1929.

3 (a) Perla, D., and Gross, H. Am J Path 11 93, 1935. (b) Reiman, H. A., Koucky, R. F., and Eklund, C. M. ibid 11 977, 1935. (c) Koletsky, S., and Stecher, R. M. Arch Path 27 267, 1939. (d) Lindsay, S., and Knorp, W. F. ibid 39 315, 1945.

is irregular and capricious<sup>4</sup> In some cases the material shows an affinity for one or more of these dyes, in others it fails to do so Even in tissues from the same patient, areas otherwise morphologically identical may fail to show the same reaction to these tests<sup>5</sup> This phenomenon has suggested the existence of a preamyloid stage of development<sup>6</sup> or the presence of multiple types of protein which are being identified collectively as amyloid<sup>5</sup>

To date, some 50 cases of primary systemic amyloidosis have been recorded in the literature On 48 of these autopsy reports are available Koletsky and Stecher<sup>3c</sup> and Lindsay and Knorp<sup>3d</sup> identified, discussed and tabulated the findings in 40 cases reported up to 1945 In 1946 Lindsay<sup>7</sup> discussed 4 additional cases, including 2 which had previously been overlooked, and added 1 of his own During and subsequent to 1946 reports of 5 additional cases, not included in the foregoing reviews, have appeared in the literature<sup>8</sup> The frequency with which such reports have been made during the past three years indicates that this disease may not be quite so rare as was previously thought An additional case is now reported

#### REPORT OF CASE

A 39 year old married white woman entered Oliver General Hospital on Feb 5, 1948 and died eighteen days later On admission to the hospital she complained of weakness of the right arm and the right leg She stated that eight days before admission, after retiring for the night, she suddenly found herself unable to speak, her right extremities were limp and could not be moved She regained her speech the following day Use of the extremities gradually returned so that at the time of admission she could walk without difficulty, although the arm and leg were still weak

The family history was noncontributory The father, the mother and ten siblings were all living and in good health One sister died of uterine cancer at the age of 47

Except for typhoid fever at the age of 6, the patient recalled no significant illnesses until 1936, when the last of six children was born This child was still-born, and the labor lasted about forty-two hours, terminated by forceps On the ninth postpartum day thoracic pains of pleuritic type developed, with fever and cough This illness followed a protracted course, with exacerbation of the thoracic pain after the second week and swelling of both legs after the third week She was hospitalized for one month and remained in bed at rest for a second month

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4 (a) Kerwin, A V J Lab & Clin Med **22** 255, 1936 (b) Eisen, H N Am J Med **1** 144, 1946 (c) Golden, A Arch Int Med **75** 413, 1945

5 Iverson, L, and Morrison, A B Arch Path **45** 1, 1948

6 Eklund, C Bull Staff Univ Minnesota Hosp **5** 180, 1934, cited by Iverson and Morrison<sup>5</sup>.

7 Lindsay, S Am Heart J **32** 419, 1946

8 (a) Orloff, V, and Felder, L Am J M Sc **212** 275, 1946 (b) Lindsay, S Am J Med **4** 765, 1948 (c) Golden<sup>4c</sup> (d) Iverson and Morrison<sup>5</sup>



Following this illness, she continued to have some intermittent swelling of the feet on prolonged standing, weakness, nervousness and fainting spells, however, she was able to hold a strenuous job in an aircraft factory for several years during the war

In 1942 she began to have epigastric aching related to meals, relieved by soda, soft drinks and food. This continued until the time of admission, with some exacerbation during the year prior to admission. For two years there had been gradually increasing dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea and edema of the legs, which at the time of admission extended above the knees. One year prior to admission she was told by a physician that her heart and liver were enlarged. For the last four months there was an intermittent pain of cutting type in the right upper quadrant of the abdomen. For three months she had noted that her abdomen was enlarging although she was losing weight. There had been a total loss of about 50 to 60 pounds (22.5 to 27 Kg.) in the one and one half year period preceding admission.

Examination revealed a well developed 39 year old white woman with pale, ashen complexion. She was apathetic, but appeared in no acute distress. The skin of her feet, ankles and wrists was thickened, scaly, slightly reddened and tender to pressure. There was pitting edema to the middle of both legs. There was marked gingivitis. The tongue was beefy red, motility was normal. There were coarse crepitant rales in both pulmonary bases posteriorly. Her heart was enlarged to the left, the tones were distinct, but no murmurs were heard. The blood pressure in the right arm was 105 systolic and 80 diastolic, that in the left arm, 108 systolic and 76 diastolic. There were infrequent premature beats. The circulation time, arm to tongue, with sodium dehydrocholate, was 27 seconds. The venous pressure was 23 cm. of isotonic solution of sodium chloride. The abdomen was protuberant in contrast to the wasting of the remainder of the body. A distinct, sharp edge of the liver, hard, apparently nodular and nontender, was palpable below the level of the umbilicus. The abdomen was distended and tympanitic. The spleen was not palpable. The neurologic examination revealed a hyperactive right knee jerk, the remainder of the peripheral reflexes were hypoaactive (but present). Babinski, Oppenheim and Romberg signs were not present. There was no evidence that cranial nerves were involved. The muscular strength of the arms and legs was equal bilaterally.

Urinalyses showed leukocytes intermittently with occasional erythrocytes, the albumin content varied from none to 2 plus. The white blood corpuscle count on admission was 18,400, with neutrophilic granulocytes 71, lymphocytes 27 and eosinophilic granulocytes 2 per cent, it varied from 10,000 to 17,000 during the hospital course, with a moderate shift to the left.

Other laboratory results were

Hematocrit reading, 42 per cent

Sedimentation rate, 16 mm per hour (Wintrobe, corrected)

Prothrombin concentration, 100 per cent

Cephalin-cholesterol flocculation (Hanger's test), negative

Thymol turbidity test, negative

Quantitative van den Bergh test—1 minute 0.2 mg, total 0.5 mg, per hundred cubic centimeters of serum

Blood proteins

Albumin 3.23 Gm

Globulin 2.15 Gm

Total 5.38 Gm

} per hundred cubic centimeters

Blood urea nitrogen, 11 mg per hundred cubic centimeters

Spinal fluid

Sugar 50 mg

Protein 15 mg

Chlorides 700 mg

} per hundred cubic centimeters

Wassermann and colloidal gold tests, negative

Repeated electrocardiograms showed low voltage QRS complexes and flattening to inversion of T waves in leads I, II and III. R waves were absent in CR 1 to 3, and T waves were inverted in CR 5 and 6 (fig 1)

In roentgen studies, the heart was diffusely enlarged in the anterior-posterior view. In the oblique views there was some impingement on the retrocardiac space

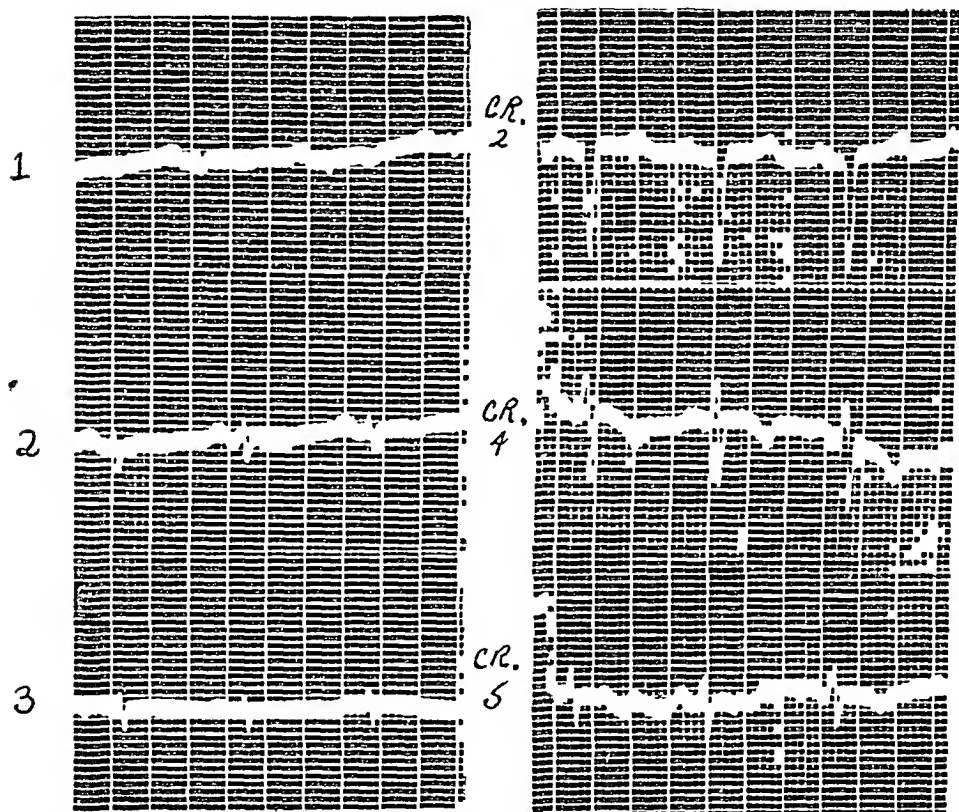


Fig 1—Electrocardiogram taken Feb 10, 1948. Note the low voltage of QRS and flattening to inversion of T waves in limb leads. R is absent in CR2, and T waves are inverted in CR4 and 5.

Under fluoroscopic examination the amplitude of pulsations appeared somewhat diminished. The great vessels did not appear dilated. It was the impression of the radiologist that no great amount of fluid was present in the pericardial sac, but the presence of some fluid could not be excluded. The general picture appeared consistent with avitaminosis or, possibly, myxedema. The lower lung field showed some haziness, believed to be due to congestion. A gastrointestinal series showed only a dilated duodenal cap with a questionable pseudodiverticulum on the side of the greater curvature.

*Course in Hospital*—The patient was placed in bed at rest and was supplied with a low salt, low fat, high carbohydrate, high protein diet with supplementary

vitamins consisting of 10 mg of thiamine hydrochloride, 2 mg of riboflavin, 100 mg of nicotinamide, 100 mg of ascorbic acid and one vitamin A-D capsule three times daily. She improved considerably during the first week in that her shortness of breath disappeared, as did the beefy redness of her tongue. The skin of the ankles and wrists became more normal in appearance, and the tenderness to pressure disappeared. On February 10 she was allowed bathroom privileges. During the following week she remained fairly comfortable, complaining mainly of nagging abdominal discomfort and occasional dizzy spells. There was no decrease in the hepatomegaly.

During the night of February 19 there suddenly developed massive edema of the lower extremities, the blood pressure fell to 90 systolic and 60 diastolic, the pulse became thready, and the patient was disoriented. On the following day the edema had decreased. She was semistuporous and showed a lag of the facial musculature with ptosis of the right eyelid. There was complete motor paralysis of the right arm and leg with hyperactive reflexes, ankle clonus and Babinski's great toe reflex on that side. A spinal tap revealed a pressure of 270 mm, rising to 350 mm on pressure of the jugular veins. The cerebrospinal fluid was clear, with sugar 50 mg, chlorides 700 mg and total protein 15 mg per hundred cubic centimeters. A tentative diagnosis of thrombosis of the left middle cerebral artery was made.

The cardiac enlargement appeared somewhat increased, but a pericardial tap showed no evidence of fluid. The patient was digitalized without notable improvement. The following day mental depression increased and Cheyne-Stokes respiration supervened. She was placed in an oxygen tent but grew progressively weaker in spite of intensive supportive therapy and died during the night of February 23.

A necropsy was made thirty-three hours after death, after contact with next of kin. The body was that of a well developed, moderately obese white woman, appearing approximately 45 years of age. The abdominal wall was flabby, with numerous silvery gray cutaneous striae, especially in the lower quadrants. The right calf appeared slightly larger in diameter than the left, there was minimal edema of the extremities. Within the abdominal cavity, approximately 350 to 400 cc of clear amber-colored fluid was present, in the left upper quadrant a thin brownish material had escaped from a ragged defect in the fundus of the stomach. The left pleural cavity contained approximately 300 cc of thin, foul-smelling, chocolate brown fluid, there was extensive autolysis of the lower third of the esophagus.

The heart weighed 600 Gm. Its contour was globoid, the chambers on the right side of the heart were prominent. The epicardial surface was smooth throughout, and the coronary vessels were readily followed beneath the epicardium by inspection and palpation. Within the chambers of the heart, the most prominent alteration was seen in the left atrium (fig 3B). Here the endocardium was diffusely thickened, numerous small semitranslucent grayish tan plaques approximately 0.2 cm in diameter were slightly upraised above the surface of the endocardium. These were distributed diffusely over the entire endocardial surface, they were more prominent in the area immediately superior to the posterior cusp of the mitral valve. The wall of the left atrium appeared definitely thicker than that of the right, but it did not appear especially stiffened. The endocardium of the remaining cardiac chambers was essentially normal in gross appearance. The cardiac valves were thin, and no nodularity was noted in the leaflets. The pulmonic valve ring was mildly to moderately dilated, it measured 9.2 cm in circumference. The measurements of the remaining valve rings in centimeters were as follows: mitral valve, 10.2, aortic valve, 7.8, tricuspid valve, 12.3. The right ventricular wall

measured 0.5 cm, the left ventricular wall 2.0 cm, in thickness. Sections through the myocardium revealed mild to moderate increase in consistency, the cut surface was of a uniform reddish brown color.

The lungs were increased in weight, the right weighed 525 Gm and the left 450 Gm. The posterior aspect of the left lung had been extensively eroded and softened by gastric contents which had escaped through the esophageal perforation. With this exception, the pleural surfaces were smooth and glistening. Crepitus appeared mildly to moderately reduced throughout both lungs. On cut section a relatively dry surface was noted, showing the usual anthracotic markings. The pulmonary vessels were not unduly prominent, compression of the lungs produced no fluid from the parenchyma or exudate from the bronchi.

The spleen weighed 535 Gm, the general contour and the appearance of the capsular surface were within normal limits, it was obviously enlarged, and its consistence was definitely increased. Cut section revealed a smooth, glossy, dull red surface on which the normal structural markings were obscured, scraping produced only minimal particles of pulp.

The liver weighed 4,200 Gm. The central portion of the liver was obviously softened and yellowish, about the periphery a more normal appearance persisted. Here the structural markings suggested chronic passive hyperemia, the central portion of the hepatic lobule was accentuated by deep brown markings, while the more peripheral portion was outlined in lighter yellow-brown, giving the appearance of the so-called "nutmeg" liver.

The autolysis of the stomach and the esophagus previously referred to involved the posterior aspect of the lower third of the esophagus and the upper fourth of the stomach along the greater curvature, the margins of these defects were softened, ragged and greenish black. In these areas of autolysis the blood vessels were not spared. There was no evidence of chronic peptic ulcer.

Each kidney revealed two or three areas of ischemic infarction, these presented the typical gross appearance, with softened yellow centers surrounded by a brighter red zone of reactive hyperemia. Vascular occlusion could not be grossly demonstrated. The right kidney presented complete reduplication of pelvis and ureter, the superior and inferior renal pelves were separated by an isthmus of renal parenchyma. The separate ureters opened into the bladder by distinct ureteral orifices, 0.7 cm apart.

The brain weighed 1,360 Gm. Its general consistence was much diminished, there was a large area of well marked encephalomalacia involving the inferior surface of the left frontal and temporal lobes together with the basal ganglia on that side. The left middle cerebral artery was occluded by a thrombus extending from the internal carotid artery throughout its entire course. Similar thrombotic occlusion likewise involved the left anterior cerebral artery, extending as far rostrally as the anterior communicating artery. The left middle cerebral artery had a small fusiform dilatation adjacent to its origin from the carotid artery. Serial sectioning after two weeks' fixation in 4 per cent formaldehyde solution revealed severe softening of the entire left frontal lobe together with the basal ganglia on the left. The cerebellum, pons, brain stem and right cerebral hemisphere presented essentially normal consistency.

A single small area of hemorrhage, approximately 0.3 cm in greatest diameter, was noted in the midportion of the pons.

The adrenal glands combined weighed 21 Gm, their consistency was moderately increased, especially in view of the thirty hour lapse prior to autopsy. Transverse section revealed that the usual bright yellow outer cortical stripe had been replaced by dull grayish brown material.

*Microscopic Observations*—Heart Beneath the endocardium and the epicardium of the left atrium (fig 2*A*) there appeared bands and masses of amorphous or finely fibrillar material, which in sections stained with Harris' hematoxylin and eosin presented a hyaline appearance, sections stained with crystal violet revealed the reddish purple metachromatic staining characteristics of amyloid. This metachromatic staining reaction was by no means uniform throughout the subendocardial deposit, it appeared in patchy areas separated by other zones in which typical metachromasia was not noted. Within the myocardium, extensive similar amyloid deposits were detected as hyaline eosinophilic material in the hematoxylin-



Fig 2—*A*, endocardium of the left atrium ( $\times 100$ ). Note the thickening of the endocardium and the irregular deposits of amyloid, which appears as denser material.

*B*, myocardium of the left ventricle ( $\times 100$ ). Note the thin amyloid rings about the muscle fibers, crystal violet stains show irregular metachromasia. Note the thickening of small arteries caused by presence of amyloid.

The photomicrographs in this and the following figure are from tissue fixed in 10 per cent formaldehyde solution, embedded in paraffin and stained with hematoxylin and eosin.

eosin section, a finding confirmed by crystal violet stains. Amyloid deposits varied from narrow rings about the individual muscle fibers (fig 2*B*) to larger strands and masses (figure 3*A*) deposited between the muscle bundles. In its most extreme form the amyloid deposit totally replaced the muscular tissue. In the areas of

minimal deposition the histologic appearance suggested that the amyloid substance made its first appearance as a membrane-like deposit at the surface of the cardiac muscle fiber. In some areas the continuity of the muscle fibers appeared to have been interrupted by masses of amyloid, but it was impossible to determine that this appearance did not indicate that the muscle fibers had deviated from the plane of section rather than that the cytoplasm had been replaced by infiltrating amyloid. Prominent throughout all sections were amyloid deposits present within the media of the small branches of the coronary arteries. They appeared as small nodular masses between the muscle fibers or replaced them, in some instances, amyloid replacement had proceeded to such an extent as to occlude the lumen of the vessel. The amyloid deposited within the media of the arterial walls almost universally

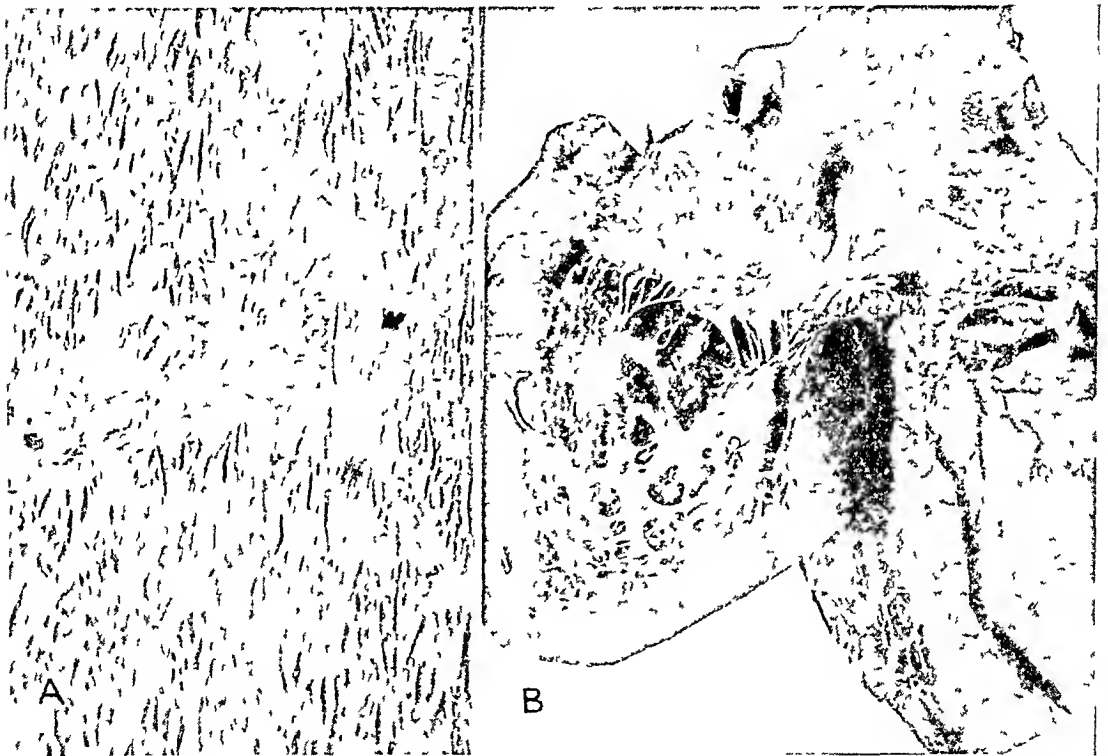


Fig 3—*A*, myocardium of the left ventricle ( $\times 100$ ). Massive intercellular deposits of amyloid are seen.

*B*, heart sectioned to show the left atrium and ventricle. The photograph is from the gross specimen, which has been preserved in Kaiserling's solution. Note the translucent subendocardial deposit in the atrial wall.

gave the metachromatic staining reaction with crystal violet. The infiltrating substance noted about and between the muscle fibers yielded a reaction similar to that observed beneath the atrial endocardium. Patchy areas revealed typical reddish purple metachromatic staining with crystal violet, while in adjacent zones this reaction failed to appear. Such irregular staining was seen alike in the areas of massive amyloid infiltration of the myocardium and in those zones of minimal deposit in which the substance appeared as a delicate layer on the surface of the muscle fibers. The deposition of amyloid was noted both within the small arteries lying beneath the epicardium and in those deep within the myocardium, certain

small branches of the cardiac veins displayed minimal deposition of amyloid within the adventitia. Examination of the subepicardial fat did not reveal pericellular deposits of amyloid.

**Lungs** The smaller branches of the pulmonary artery and the pulmonary arterioles showed the media replaced to varying degrees by amorphous masses of hyaline, deeply eosinophilic material, which in sections stained with crystal violet failed to give the typical metachromatic staining reaction. Sections stained with phosphotungstic acid-hematoxylin differentiate this substance from collagen. In addition to this vascular infiltration small strandlike deposits of amyloid were noted within the interalveolar septums, in this location they paralleled the course of the precapillary arterioles and that of the capillaries. This material gave the staining reactions previously described. In several areas beneath the pleura, small triangular infarct-like areas of necrosis of the interalveolar septums were associated with intra-alveolar hemorrhage. Adjacent to such areas there was nearly always a small branch of the pulmonary artery in which the lumen had been completely obliterated by massive medial deposition of amyloid.

In addition to the amyloidosis described to this point there were small focal areas of bronchopneumonic infiltration of groups of alveoli adjacent to the bronchioles. Scattered "heart failure" cells were noted within some of the alveoli.

**Spleen** The splenic parenchyma was almost totally replaced by masses of amyloid, which appeared to be deposited beneath the endothelium lining the splenic sinusoids. The sinusoids were markedly compressed, and the splenic cords were almost totally replaced. The malpighian corpuscles were so reduced in size and number that they were identified only with difficulty. Sections stained with crystal violet gave only a faintly metachromatic reaction, sections stained with phosphotungstic acid-hematoxylin revealed the infiltrating substance, stained reddish orange, which differentiated it sharply from normal connective tissue and muscle elements. The central arterioles of the splenic corpuscles revealed nodular deposits of amyloid replacing their media. The infiltrate here, in contrast to that noted within the splenic pulp, stained metachromatically with crystal violet.

**Liver** Large amounts of amyloid material were deposited between the sinusoidal endothelium and the underlying liver cells, there was total obliteration of the normal hepatic structure in the central and midzones of the lobules, about the periphery a semblance of normal structure persisted. There was extensive medial amyloidosis of the small arteries throughout the section. Here, as in the spleen, the amyloid deposited within the walls of the blood vessels stained metachromatically with crystal violet, while that deposited beneath the sinusoidal endothelium failed to give this reaction.

**Genitourinary System** The renal amyloid deposit was restricted to small nodular masses deposited beneath the endothelium of the glomerular capillaries, in no instance did this progress to complete obliteration of a glomerulus. The amyloid here failed to stain metachromatically. In contradistinction to the glomeruli, the renal vascular system showed fairly impressive medial amyloidosis of the arteries. Metachromatic staining was noted in some cases, while it was absent in others, vessels showing typical eosinophilic hyaline material in the section stained with hematoxylin and eosin but failing to show metachromasia in the section stained with crystal violet were usually the smaller branches of the renal artery. Sections taken through the margin of one of the grossly described infarcts revealed a typical histologic appearance with palely eosinophilic "ghosts" of renal structures. A small nodular deposit of amyloid was noted within the renal capsule in one section.

The uterine sections showed extensive amyloid replacement of the myometrium, the amyloid stained metachromatically. There was also medial amyloidosis in a few of the arteries of the myometrium.

**Ovaries** Ovarian sections revealed amyloidosis confined to the media of the smaller arteries, metachromatic staining of the amyloid was noted.

**Adrenal Gland** Sections of adrenal gland showed massive amyloidosis of the cortex, the change appeared most marked in the zona fasciculata, though less prominent changes were noted in the zona glomerulosa and the zona reticularis. In the cortex, the histologic structure suggested that the amyloid had been deposited beneath and between the epithelial cells of the adrenal cell cords. The epithelial cells were displaced into the sinusoids, with obliteration of the last-named structures in many instances. In medium and smaller-sized arteries beneath the adrenal capsule there was extensive medial deposition of amyloid. In the periadrenal fat the crystal violet stain gave a typical amyloid reaction in only small, scattered areas.

**Central Nervous System** Sections of the brain showed the typical degenerative changes of extensive anemic softening in the areas grossly involved in the encephalomalacic process. Sections through the left midcerebral artery revealed occlusion due to a laminated thrombus of the antemortem type, section through the grossly described aneurysmal dilatation revealed arteriosclerotic changes within the wall of the artery. Phosphotungstic acid-hematoxylin revealed splitting of the internal elastic lamina with deposition of a fibrillar material having the staining affinities of collagen between the separated layers. There was also subintimal deposition of fibrillar amorphous substance which appeared to be atheromatous in nature. Certain sections revealed lamination of the vessel wall, the split occurring external to the internal elastic lamella. There is no evidence of amyloid deposition within the vessel wall.

**Miscellaneous Sites** In addition to the deposits described, small deposits of amyloid were found in the media of the small and medium-sized arteries and arterioles of the duodenum, the ileum, the uterine tube, the pancreas and the bone marrow. The esophagus showed acute inflammation with edema of the wall and chronic inflammatory cell infiltration.

**Pathologic Diagnoses**—Primary systemic amyloidosis involving the interstitial tissues of the heart, the liver, the spleen, the adrenal glands and the uterus, as well as small and medium-sized arteries of these organs, together with those of the kidneys, the ovaries, the uterine tubes, the gastrointestinal tract, the lungs, the pancreas and the bone marrow, ischemic infarction of the left frontal lobe of the cerebrum, together with the basal ganglions on the left, secondary to arteriosclerotic thrombosis of the left midcerebral artery, focal bronchopneumonia, double right kidney and ureter, acute esophagitis, esophagomalacia and gastromalacia, multiple renal infarcts, bilateral, multiple small areas of pulmonary infarction.

#### COMMENT

This case fulfils the most important criteria of primary systemic amyloidosis in that there is no evidence of any pre-existing condition recognized as capable of bringing about the formation of amyloid. The irregular and inconstant metachromatic staining with crystal violet is in keeping with observations of others, the subendocardial and myocardial deposits of amyloid in no way differ from those described in



many previous instances and reviewed by Lindsay<sup>7</sup> The appearance of extensive deposition of amyloid in the liver, the spleen and the adrenal glands is less characteristic Of the 22 cases reviewed by Koletsky and Stecher<sup>3c</sup> as instances of primary systemic amyloidosis, involvement of the spleen was observed in 2, adrenal involvement in 2 and hepatic deposition of amyloid, except for that found within the blood vessels, in none The group of 16 cases reviewed by Lindsay and Knorp<sup>3d</sup> included 8 in which the hepatic parenchyma, 7 in which the spleen and 6 in which the adrenal glands were involved This overlapping of distribution, involving sites characteristic of secondary amyloidosis, has been previously commented on<sup>9</sup> Conversely, instances of amyloidosis of the secondary type have been reported in which the major sites of involvement were in the tissues of mesodermal origin, the secondary distribution simulating that seen in primary systemic amyloidosis<sup>10</sup>

Pericellular deposits of amyloid occurring in adipose tissue have been described by Peters,<sup>11</sup> Iverson and Morrison<sup>5</sup> and Pearson and co-workers<sup>12</sup> Peters considered such deposits to be the earliest form in which amyloid appeared No evidence of such distribution has been found in adipose tissue in this case In the myocardium, however, thin amyloid shells were present about the muscle fibers in the areas of least marked involvement (fig 2*B*) Larsen<sup>13</sup> concluded, from his study of serial sections, that amyloid is primarily a pericapillary deposit spreading into the interstitial tissue In the case which he reported, there was no involvement of the coronary arteries, and he felt that the deposition of amyloid depended on changes of the permeability of the venous endothelium, since he often found amyloid in the myocardial venules In our case, this distribution is reversed, and we find extensive amyloidosis of the coronary arteries with only minimal involvement of the cardiac veins This question of the sites of the earliest or the most prominent deposition of amyloid is of some importance, for the answer has a bearing on the problem of the origin of the substance Peters<sup>11</sup> cited German and Dutch authors of the early decades of this century, who are said to have described epicellular deposits of amyloid without having questioned the older doctrine that the material was formed as a transudate He pointed out that deposition of amyloid occurred in the walls of arteries and referred to the difficulty of accounting for

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9 Reiman and others<sup>3b</sup> Koletsky and Stecher<sup>3c</sup> Orloff and Felder<sup>8a</sup> Golden<sup>4c</sup> Iverson and Morrison<sup>5</sup>

10 (a) Spam, D M, and Barrett, R C Arch Path **38** 203, 1944 (b) Budd, J W Am J Path **10** 299, 1934 (c) Lindsay and Knorp<sup>3d</sup>

11 Peters, J T Arch Path **35** 832, 1943

12 Pearson, B, Rice, M M, and Dickens, K L Arch Path **32** 1, 1941

13 Larsen, R M Am J Path **6** 147, 1930

a transudate in such an area in support of his contention that amyloid arises at the surfaces of cells. Larsen<sup>13</sup> noted medial amyloidosis of the aorta, the pulmonary vessels and the inferior vena cava, but he explained these deposits as related to the vasa vasorum, in accordance with his belief that amyloid was deposited from the intercellular fluid ("tissue lymph") as a result of altered venous permeability.

Mallory<sup>14</sup> held that amyloid was formed by perverted fibroblastic activity and that its being deposited immediately beneath vascular or sinusoidal endothelium was related to the fact that there were fibroblasts in the vicinity. Warren<sup>15</sup> supported and elaborated this contention, stating in connection with his report of a case of generalized muscular amyloidosis that the condition represented a widespread perversion of function of connective tissue elements of muscular structures of the body, involving smooth, striated and cardiac muscle. In his opinion there was no doubt that this was the mode of origin, for in many instances amyloid was found at a considerable distance from blood vessels.

It is manifestly impossible to draw conclusions regarding the early localization of amyloid from massive deposits such as are seen in the liver, the spleen and the adrenal glands in this case, as well as the relatively heavy accumulations in some portions of the myocardium and beneath the endocardium. The pericellular amyloid rings that we and others have seen are in keeping with the hypothesis that amyloid is produced by local cellular activity and tend to support the beliefs of Peters,<sup>11</sup> Mallory<sup>14</sup> and Warren<sup>15</sup> rather than the theories of those<sup>16</sup> who hold that amyloid is an abnormal protein precipitated from the body fluids.

In our case the chief feature of interest is the association of widespread amyloidosis of the cardiovascular system with massive deposits of amyloid in the liver, the spleen and the adrenal glands. This reemphasizes the fact that distribution alone does not differentiate primary from secondary systemic amyloidosis.

Of incidental interest is the autolysis of the fundus of the stomach and the lower part of the esophagus. This may be associated with the intracranial lesion.<sup>17</sup>

Many systems may be involved in primary amyloidosis, but they are seldom involved to a uniform extent. The pathologic change is frequently overshadowing in, and the symptom complex primarily

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14 Mallory, F. B. *Principles of Pathologic Histology*, Philadelphia, W. B. Saunders Company, 1914 (reprinted 1925).

15 Warren, S. *Am J Path* 6:161, 1930.

16 Perla and Gross<sup>3a</sup>; Larsen<sup>13</sup>.

17 Moore, R. A. *Textbook of Pathology*, Philadelphia, W. B. Saunders Company, 1945.

referable to, the cardiovascular system, the gastrointestinal tract, the lungs, the genitourinary system, etc. In the 50 cases previously reported an extensive involvement of the cardiovascular system has been common. In 24 cases there were clinical evidences of heart failure prior to death. In 21 of 48 fatal cases heart failure was indicated as a cause of death. Some degree of amyloid infiltration of the heart was found in 43 of 48 cases studied at autopsy.

Lindsay,<sup>7</sup> in his discussion of the heart involved in primary amyloidosis, lists the following mechanisms leading to heart failure:

1. Involvement of the pulmonary vessels and alveolar walls leading to chronic cor pulmonale (dilatation of the heart)

2. Amyloidosis of the cardiac vessels leading to coronary insufficiency or infarction

3. Interstitial amyloid infiltration of the myocardium with or without secondary degeneration of muscle fibers

4. Pericardial or endocardial deposition of amyloid

5. Amyloid involvement of cardiac valves, with stenosis or insufficiency

6. Combinations of the aforementioned mechanisms

It would be expected that electrocardiographic findings would be variable depending on which of the aforementioned factors were dominant in the production of the heart failure. Diffuse infiltration of the myocardium would interfere with the strength of contraction and the flow of current through the heart walls. Detailed electrocardiographic reports are available in only 13 recorded cases. In 9 of these, heart failure was listed as a cause of death. Low voltage of the QRS complexes was the prominent feature in 7 cases and flattening or inversion of T waves in 2 cases.

In this case the cerebrovascular lesions were found to be due to thrombosis of arteriosclerotic vessels and were not related to the amyloid disease. The clinical picture was primarily that of cardiac failure of obscure cause, correlated at autopsy with amyloidosis of the myocardium. The abdominal enlargement with the grossly enlarged, hard liver and the globular-shaped heart led to consideration of mediastinopericarditis with pseudocirrhosis of the liver (Pick), although there was no evidence of constriction or fixation of the heart. The electrocardiogram showed low voltage and flattening of the T waves, these were the changes most prominent in previously reported cases of cardiac amyloidosis in which such studies were made.

The beefy red tongue and the cutaneous changes that were noted at the wrists and ankles of this patient were assumed clinically to be due to a vitamin deficiency, since there had been a poor nutritional

intake for a long period prior to hospitalization. Credence was lent to this view because of the definite and rapid improvement which occurred in these lesions under intensive vitamin therapy. Unfortunately, no sections were taken from these sites for microscopic study, since no gross abnormality could be noted at the time of autopsy and the nature of the general systemic disease was not then suspected. Amyloid involvement of the skin and the tongue has not been uncommon in the previously reported cases, whether there was such involvement in this patient and whether that involvement contributed to the clinical findings must remain a question.

It appears that this case further substantiates the observation of Lindsay<sup>18</sup> that primary systemic amyloidosis should be considered in the differential diagnosis of cardiovascular disease whenever the cause is obscure and the symptom complex and the clinical findings bizarre.

#### SUMMARY

Primary systemic amyloidosis is becoming more frequently recognized as a clinical entity. Its causation will presumably remain a mystery until the disputed chemical nature of amyloid is resolved. The literature is briefly reviewed, and an additional case is reported. Primary amyloidosis of the cardiovascular system should be considered in the presence of bizarre symptoms and findings of obscure cause, especially when the electrocardiogram indicates diffuse myocardial involvement.

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18 Lindsay and Knorp<sup>3d</sup> Budd<sup>10b</sup>

# MECKEL'S DIVERTICULUM WITH ABERRANT PANCREATIC TISSUE AND A POLYP CONTAINING GASTRIC GLANDS

M C WHELOCK, M D

AND

H A TELOH, M D

CHICAGO

OF THE pathologic conditions due to congenital diverticulum of the small intestine, those associated with aberrant or heterotopic tissue are of the greatest interest. The occurrence of gastric mucosa, pancreatic tissue with or without islets of Langerhans, duodenal mucosa or colic mucosa is well known and has been frequently reported. In 1934 Hunt and Bonestiel<sup>1</sup> gave an excellent review of the occurrence of aberrant pancreatic tissue in the gastrointestinal tract including Meckel's diverticulum. However, the occurrence of a polyp in Meckel's diverticulum is rare, and only sporadic reports of it are found in the literature. The case reported now is considered of pathologic interest.

## REPORT OF A CASE

A 51 year old white man was first admitted to Passavant Memorial Hospital, July 14, 1948, for bleeding gums, easy bruising, and numerous spots on his hands, arms and legs, present for three months. He had also noticed easy fatigability on exertion for a similar period. He had pneumonia in 1933 and underwent left herniorrhaphy in 1928.

The patient was well developed and well nourished. The temperature was 101 F, the pulse rate, 80, the respiratory rate, 20, and the blood pressure 120 systolic and 70 diastolic. There were petechiae on the pale blue boggy buccal mucosa, an ulcerated area on the upper left gum and blood oozing from around the base of the lower left canine tooth. The lower extremities were covered with small red macular lesions and ecchymoses.

The red blood cell count was 2,540,000, the hemoglobin content, 10 Gm. The white blood cell count was 2,800, with 3 band cells, 21 segmented cells, 1 eosinophilic granulocyte, 71 lymphocytes, 2 myelocytes, 1 metamyelocyte and 1 monocyte. The specific gravity of the urine was 1.017,  $pH$ , 6.0, albumin, a trace, sugar, none, red blood cells, 50 to 60, leukocytes, 20 to 30. The thrombocyte count was 7,620, the prothrombin time, 101.7 per cent. The blood urea nitrogen was 12.4 mg, blood sugar, 108 mg, blood ascorbic acid, 1.42 mg, serum total protein 6.04 Gm, albumin, 3.67 Gm, globulin, 2.37 Gm, per hundred cubic centimeters.

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From the Department of Pathology of Northwestern University Medical School and Passavant Memorial Hospital.

1 Hunt, V. C., and Bonestiel, H. T. S. Arch Surg 28:425, 1934.

A diagnosis of primary aplastic anemia was made

On July 25, 1948 the patient was readmitted on account of marked pyuria. Cystoscopic and retrograde pyelographic examination led to a diagnosis of bilateral hydronephrosis and bilateral ureteral stricture with an enlarged prostatic bar. Postoperatively, there was profuse bleeding from the urinary tract. After five days this was controlled with blood transfusions.

The patient was readmitted on two subsequent occasions for blood transfusions. The blood picture remained unchanged.

Seven days after the date of the patient's fourth discharge, diarrhea developed, with four black stools during the day. Seven similar stools occurred on the following day.

The patient reentered the hospital on November 19. He was pale and listless. The oral temperature was 99 F, the pulse rate, 112, the respiratory rate, 20, and the blood pressure, 104 systolic and 60 diastolic. Clotted blood was seen about the upper molar and lower incisor teeth, dried blood on the dorsum of the tongue and a small amount of blood with ecchymoses in the oropharynx. There were multiple petechiae and ecchymoses of the face. The abdomen was soft and nontender, and no organs or masses were palpable. Otherwise the examination disclosed nothing of importance.

The red blood cell count was 1,520,000, the hemoglobin content, 40 Gm. The white cell count was 1,550. No thrombocytes were seen. The specific gravity of the urine was 1.018,  $pH$ , 6.0, albumin, 1 plus, sugar, none. The stool was black to dark red and gave a strongly positive reaction to the guaiac test. The patient received multiple massive transfusions over a period of twenty-four days, but severe intestinal hemorrhage continued. Surgical intervention was considered, but at no time was the patient considered to be in good enough condition to risk operation. He died twenty-four days after admission, of a massive hemorrhage of the rectum.

The anatomic diagnoses were: diverticulum of the small intestine with ulceration and acute and chronic inflammation, pedunculated polyp of the small intestine with aberrant gastric mucosa, aberrant pancreatic tissue in the diverticulum of the small intestine, hypoplasia of the bone marrow, marked, subdural hematoma, bilateral, intracerebral hemorrhages, multiple, acute passive hyperemia of the lungs, liver, spleen and kidneys, bilateral hydronephrosis and hydroureter, severe, chronic cystitis, hydrohemopericardium, chronic cholecystitis, cholelithiasis, generalized arteriosclerosis, slight, emphysema of the lungs, moderate, atelectasis of the lungs, chronic focal esophagitis, fibrosis of the testicles.

Sixty centimeters proximal to the ileocecal valve Meckel's diverticulum was encountered on the antimesenteric border of the ileum. This diverticulum measured 8 cm in length and 3 cm in diameter. The external surface was mottled gray to purple. On section the wall was thick and indurated and the mucous membrane deep purple to black. There were multiple areas of superficial ulceration of the mucous membrane. At the opening of the diverticulum there was a soft, friable polyp, 2.5 cm in length and 0.8 cm in maximum diameter. The surface was deep red to black and hemorrhagic in appearance. The intestinal contents proximal to this point were normal. Distally the contents were black and putty-like. There were numerous areas of ecchymoses scattered throughout the entire intestinal tract.

In microscopic sections through the diverticulum, the layers normally found in the small intestine were all present. Focally the muscularis was greatly attenuated or absent. There were marked diffuse infiltration of all layers with

acute and chronic inflammatory cells and diffuse interstitial edema, fibrosis and numerous focal areas of hemorrhage. The interstitial hemorrhage was most marked in the submucosa. The mucosa was partly autolyzed, and there were multiple focal areas of superficial ulceration. The base of the ulcerated areas was formed of necrotic debris, a thick layer of fibrin and polymorphonuclear leukocytes. In sections of the diverticulum there were small nodular areas in

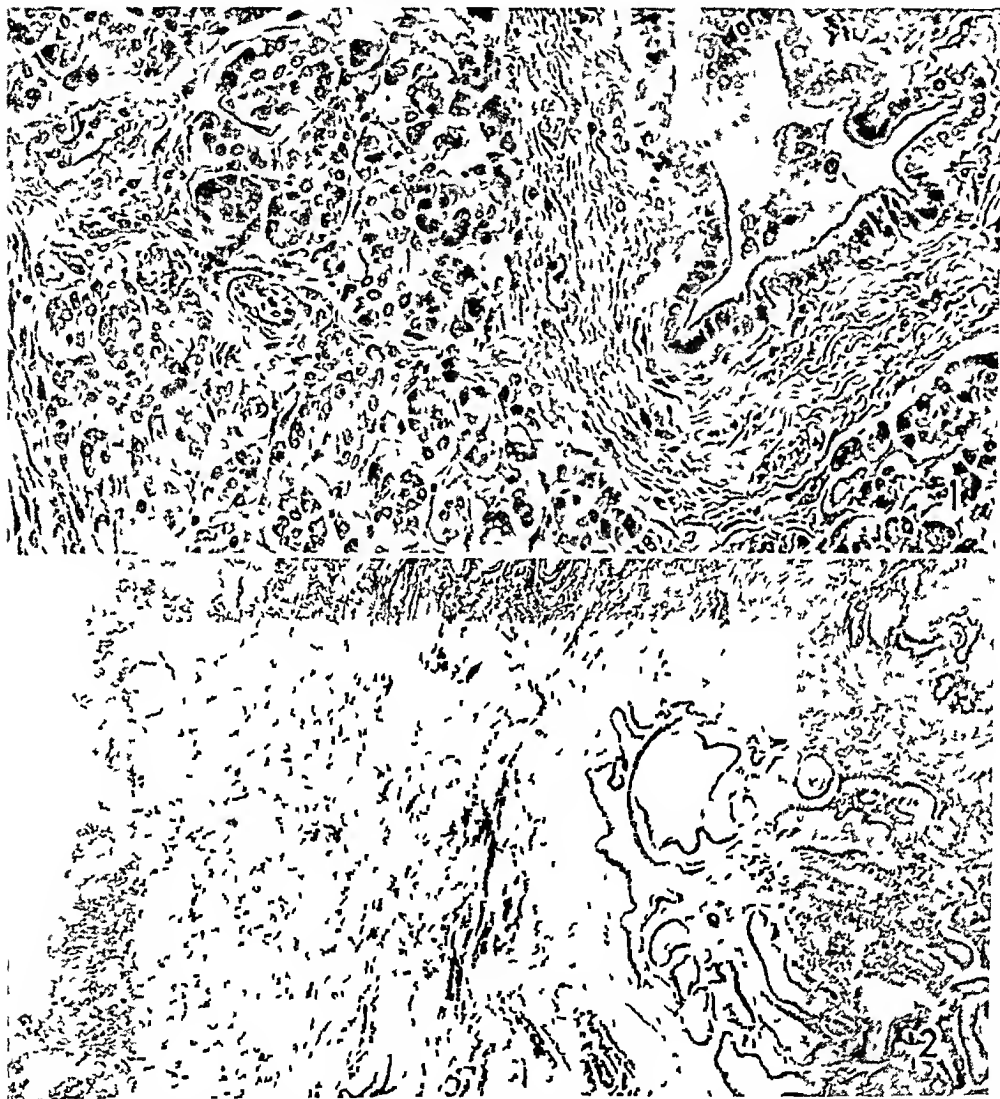


Fig 1—Pancreatic tissue in the submucosa of the diverticulum.  $\times 90$

Fig 2—Gastric mucosa at the tip of the polyp and dilated, distorted gastric glands in the stromal tissue.  $\times 90$

the submucosa, composed of pancreatic tissue, the largest of these measured 4 mm in maximum diameter. There were no islets of Langerhans.

The section of the polyp consisted of an overlying mucosa and a dense fibrous stroma containing numerous glandular structures. The mucosa consisted throughout most of the surface of an intestinal type of glandular epithelium which was continuous with that of the small intestine and diverticulum. In this

mucosa there was a marked chronic inflammatory reaction with focal areas of interstitial hemorrhage. Around the tip of the polyp the mucosa was composed of typical gastric glands and also contained numerous chronic inflammatory cells and areas of hemorrhage. The gastric glands extended into the stroma for a considerable distance to form small and large, irregularly shaped and occasionally dilated glandular structures. The stroma consisted of a dense fibrous connective tissue with numerous focal areas of hemorrhage and marked diffuse infiltration with chronic inflammatory cells.

#### COMMENT

Meckel's diverticulum occurs in approximately 2 per cent of persons coming to autopsy. It occurs as a finger-like projection from the anti-mesenteric border of the ileum from 25 to 100 cm proximal to the ileocecal junction. It may vary considerably in size, from a small flattened pouch of the ileal wall to a large tubular structure 10 cm or more in length. Its diameter usually approximates that of the ileum.

Embryologically it represents a failure of obliteration of the fetal omphalomesenteric duct or vitellointestinal duct which forms the connection between the primitive alimentary canal and the yolk sac. Normally this becomes obliterated at the seventh week of fetal life. The presence of polyps in this region is unexplained. The fact that these polyps are frequently composed wholly or partly of heterotopic tissue would seem to exclude their fortuitous occurrence in Meckel's diverticulum.

According to the literature, there are 14 recorded cases of Meckel's diverticulum with a polyp. Many of these were incompletely studied pathologically and are necessarily discarded. Hertzler and Gibson<sup>2</sup> reported the case of a 19 year old boy with an intussusception associated with invagination of Meckel's diverticulum. The diverticulum contained a small papillary lesion composed of Brunner's glands. In a review of cases they enumerated 8 previously recorded cases of polyps of Meckel's diverticulum. In 7 of these the structures were not studied histologically. In the eighth, that of Mathieu and Davioud, the specimen consisted of a small polyp composed of pancreatic tissue. Schullinger and Stout<sup>3</sup> reported a case of massive hemorrhage of the bowel associated with Meckel's diverticulum. The diverticulum contained a small adenoma composed of gastric and duodenal glands. In a study of the literature they failed to find a similar example of gastric and duodenal gland adenoma of a diverticulum, and cited 3 previously reported cases of polyps. That of Lecene was an instance of Meckel's diverticulum with an adenoma at the apex. This, however, was covered by mucous glands resembling normal intestinal mucosa. The case of

2 Hertzler, A. E., and Gibson, E. T. *Am J M Sc* **146** 364, 1913

3 Schullinger, R. N., and Stout, A. P. *Arch Surg* **28** 440, 1934



Bize was one of Meckel's diverticulum with a nodule at the apex which contained pancreatic glands with some portions having an adenomatous appearance consisting of large branching columnar cell-lined ducts. The third case was that of Hertzler and Gibson.

Starling<sup>4</sup> reported the occurrence of severe melena associated with a polyp. A "simple papilloma" was found a few inches beyond a diverticulum. No histologic examination is recorded. Bowen<sup>5</sup> summarized the previous literature and reported the case of a 14 year old boy with an intussusception. At operation Meckel's diverticulum with inversion was found in the affected bowel. It contained a polyp measuring 2 by 2 by 1.5 cm., also peptic glands.

In summary, of the cases found in the available literature, the following have been adequately studied histologically and are acceptable:

- 1 Mathieu and Davioud—polyp composed of pancreatic tissue
- 2 Lecene—adenomatous polyp formed of normal intestinal mucosa
- 3 Bize—nodule composed of pancreatic glands and large branching columnar cell-lined ducts
- 4 Hertzler and Gibson—papillary lesion composed of Brunner's glands
- 5 Schullinger and Stout—adenoma composed of gastric glands and Brunner's glands
- 6 Bowen—polyp containing peptic glands

*Aberrant Pancreatic Tissue*—Aberrant or accessory pancreatic tissue was first described by Klob in 1859. The first recorded case of aberrant pancreatic tissue in Meckel's diverticulum was that of Zenker in 1861. It is an infrequent anomaly. Hunt and Bonestiel,<sup>1</sup> in a review of 186 cases of aberrant pancreatic tissue, found 13 cases involving Meckel's diverticulum. In 1940 Faust and Mudgett<sup>6</sup> reviewed 370 cases of aberrant pancreas, 21 of which were examples of occurrence in Meckel's diverticulum.

Zenker expressed the belief that it is due to an additional bud from the foregut which has developed into a simple independent glandular mass and that this, as the foregut elongates, may be carried a great distance from its point of origin. In support of his theory is the fact that aberrant pancreatic tissue may be found along the entire gastrointestinal tract from the stomach to the terminal portions of the ileum, as well as in the gallbladder, splenic capsule, umbilical fistula, omentum and mesentery.

4 Starling, H. J. *Guy's Hosp. Rep.* **85** 207, 1935.

5 Bowen, F. H. *J. M. A. Georgia* **30** 390, 1941.

6 Faust, D. B., and Mudgett, C. S. *Ann. Int. Med.* **14** 717, 1940.

Various other theories have been suggested to account for the presence of gastric mucosa and pancreatic tissue in ectopic foci. Displacement of tissue during embryonic development may be due to the formation of additional anlage, with subsequent displacement along the gastrointestinal tract (Zenker's original theory), or it may be due to a transposition of tissue from the original site as a result of adhesions, either inflammatory or noninflammatory. Other authorities believe that it is due to a metaplasia of tissue during either fetal or adult life. The frequent presence of a marked inflammatory reaction would tend to support this theory. The atavistic theory supposes a reversion to a more primitive phylogenetic type as exemplified by certain lower animals and fishes. In these species one finds pancreatic tissue in the muscular coats of the intestinal tract, in the peritoneum and scattered diffusely through the intestinal tract. The theories of origin of heterotopic tissue are reviewed by Troll.<sup>7</sup>

Histologically, the aberrant tissue is identical with normal pancreatic parenchyma in structure and arrangement. Islets of Langerhans may or may not be present.

#### SUMMARY

A report of a case of massive intestinal hemorrhage complicating thrombopenia and primary refractory anemia is presented. At autopsy Meckel's diverticulum was found associated with a glandular polyp. The diverticulum contained aberrant pancreatic tissue, the polyp contained typical gastric mucosa and dilated, distorted gastric glands.

A review of the literature is presented and the acceptable reports of Meckel's diverticulum associated with a polyp are summarized.

The occurrence and the theories of origin of aberrant pancreatic tissue are briefly reviewed.

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7 Troll, M. M. Arch. Path. 38: 375, 1944.

## Notes and News

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**Research Fellowships**—The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1950 to June 30, 1951. These fellowships are designed to provide an opportunity for research training either in the basic medical sciences or in the application of these sciences to clinical investigation. They are for the benefit of physicians who are in the early stages of their preparation for a teaching and investigative career in internal medicine. Assurance must be provided that the applicant will be acceptable in the laboratory or the clinic of his choice and that he will be provided with the facilities necessary for the proper pursuit of his work. The stipend will be from \$2,200 to \$3,200.

Application forms will be supplied on request to The American College of Physicians, 4200 Pine Street, Philadelphia 4, and must be submitted in duplicate not later than October 1, 1949. Announcement of awards will be made in November 1949.

## Announcements

### THE AMERICAN CANCER SOCIETY FELLOWSHIPS IN EXFOLIATIVE CYTOLOGY Regulations for 1948-1949

At the American Cancer Society's symposium on exfoliative cytologic diagnostic technics held in Boston in April 1948, it was the opinion of the invited delegates that facilities were urgently needed for the training of qualified pathologists and clinicians in teaching positions at approved institutions providing residency training in pathology as well as facilities for training technicians.

At the same time the delegates recommended that the American Cancer Society proceed to engage actively the interest and support of institutions and laboratories in setting up such training programs where the best training facilities appeared to be available. Thus inquiries were sent to fourteen laboratories over the country, and on the basis of their interest and the monies available, grants will be made for fellowship training in ten laboratories to support twenty-three fellows.

#### PURPOSE OF TRAINING

To provide training in the diagnostic technics of exfoliative cytology for qualified pathologists. It is anticipated that trainees will not assume the role of teachers until sufficient personal experience and competence have been acquired.

#### AWARDS

Fellowships will be awarded by institutions or laboratories designated by the Society to applicants on the basis of their past training and their intention to teach in their own laboratories diagnostic technics in exfoliative cytology to interested pathologists, clinicians and technicians.

#### ELIGIBILITY OF APPLICANT

The applicant for a Fellowship in Exfoliative Cytology of the American Cancer Society shall

- 1 Be a graduate of a Class A Medical School of the United States, its Territories or Canada
- 2 Be a citizen of the United States

- 3 Be not over 50 years of age on the next birthday following commencement of fellowship tenure
- 4 Have completed two years of post-graduate training in pathology
- 5 Conform in other respects to requirements of the institution to which he applies

## TERM OF FELLOWSHIP

Each fellowship will be awarded for a period of four months. Fellowship training may commence at any time. The fellowship is not subject to renewal.

## STIPEND

The stipends shall be paid in two sums: the first, a grant to the laboratory for tuition, overhead, other expenses as outlined to the Society; the second, to the trainee to partially cover his board, room and incidentals for the period of training. The latter sum shall amount to \$140 per month and shall be paid direct to the fellow monthly in advance to avoid payment of income tax for services rendered to the hospital.

## APPLICATION

The individual applicant for a Fellowship in Exfoliative Cytology shall apply directly to the institution where a fellowship is available. In no instance shall application be made directly to the American Cancer Society.

## FELLOWSHIPS AVAILABLE

| Laboratory   | Number | Director                     |
|--|--------|------------------------------|
| Cornell University Medical College,<br>New York        | 6      | George N. Papanicolaou, M.D. |
| Jefferson Hospital, Philadelphia                       | 6      | Lewis C. Scheffey, M.D.      |
| University of Oregon Medical School,<br>Portland, Ore. | 2      | Warren C. Hunter, M.D.       |
| University of California Hospital,<br>San Francisco    | 2      | Heibert F. Traut, M.D.       |
| Michael Reese Hospital, Chicago                        | 2      | Otto Saphir, M.D.            |
| Hartford Hospital, Hartford, Conn.                     | 2      | Ralph E. Kendall, M.D.       |
| New York Post-Graduate Hospital,<br>New York           | 1      | Locke L. Mackenzie, M.D.     |
| Free Hospital for Women,<br>Brookline, Mass.           | 1      | Arthur T. Hertig, M.D.       |
| Mayo Clinic, Rochester, Minn.                          | 1      | John R. McDonald, M.D.       |

## Books Received

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CLINICAL ASPECTS AND TREATMENT OF SURGICAL INFECTIONS By Frank Lamont Meleney, M D, associate professor of clinical surgery, College of Physicians and Surgeons, associate visiting surgeon, Presbyterian Hospital, New York With a foreword by Allen O Whipple, M D Pp 840, with 289 illustrations Price \$12 Philadelphia and London W B Saunders Company, 1949

This book consists of eighteen chapters. It presents a documented history of the development of the treatment of surgical infections and emphasizes the fundamental surgical principles as well as the newer therapeutics, such as chemotherapy and the use of antibiotics. It is well illustrated with drawings, photographs and case reports. The various areas included in general surgery are discussed and also the surgical specialties. The author has made great contributions to the field of surgical bacteriology and is especially well qualified to produce a text on this subject. In addition, Dr Meleney has enlisted the help of a number of able scientists, whose investigations and experience enhance the value of this work. The presentation includes physiologic, bacteriologic and pathologic aspects of the lesions as well as important factors of both diagnosis and treatment. Because of the excellent manner in which the material is presented and the scientific background of the writers, this book will fill the long-standing need of a source of information and will occupy an unusual position. It is an excellent book for the use of both medical students and practicing surgeons.

BLOOD TRANSFUSION By Elmer L DeGowin, M D, associate professor of internal medicine, State University of Iowa, and director of the blood transfusion service of the University Hospitals, Robert C Hardin, M D, assistant professor of internal medicine, State University of Iowa, and John B Alsever, M D, senior surgeon, United States Public Health Service, and chief, Professional Standards, Hospital Division, United States Public Health Service Pp 587, with 200 diagrammatic drawings Price \$9 Philadelphia and London W B Saunders Company, 1949

It is a great pleasure to find a book in which authors and publishers have succeeded not only in satisfying the intelligence of the reader but in gratifying his artistic sense as well. It is attractively bound, is printed on good paper and is interestingly illustrated. The text includes a remarkably complete discussion of the ways in which whole blood and its constituent parts are used in the treatment of human disease. The book begins with a brief historical chapter and then proceeds immediately to a description of the therapeutic value of blood and of the blood derivatives and plasma substitutes that are available for intravenous, intramuscular and topical use. The cause and the treatment of shock are described. This is followed by an excellent section devoted to a discussion of ABO, MN, P and RhHr blood groups. The inheritance of blood groups, the occurrence and the immunizing capacity of the various antigens and the clinical aspects of immunization are handled clearly and completely.

The second section is devoted largely to technics and should prove extremely valuable to any one concerned with any part of a transfusion service. It begins with detailed, well illustrated descriptions of technics for determining antigens and antibodies and describes the other steps necessary in preparing for a trans-

tusion or in studying various pathologic states. It then gives methods for measuring the survival time of erythrocytes, for estimating bilirubin and urobilinogen in blood and excreta and for determining specific gravity and total blood volume. Chapters are devoted to donors (their selection, the technique of drawing blood, the possible complications) and to recipients (the methods by which transfusions can be given and the cause, diagnosis, prevention and treatment of transfusion reactions). Methods of storing blood and transporting it are described. The merits and drawbacks of plasma and the methods by which it can be prepared and fractionated are followed by the use of red cell suspensions, blood derivatives and plasma substitutes. The practical experience of the authors in running blood banks makes the chapters on blood banks and on community, regional and state blood services especially valuable. The book closes with descriptions of the apparatus for giving blood transfusions and for preparing fluids for parenteral therapy.

This book presents a more comprehensive survey of the theoretic and practical aspects of blood as a therapeutic agent than has ever before been available, and it should answer a growing need arising as a result of the increasing appreciation of the therapeutic value of blood and its derivatives.

**CURRENT THERAPY, 1949, LATEST APPROVED METHODS OF TREATMENT FOR THE PRACTICING PHYSICIAN.** Howard F. Conn, M.D., editor. Consulting editors: M. Edward Davis, Vincent J. Derbes, Garfield G. Dunsan, Hugh J. Jewett, William J. Kerr, Perrin H. Long, H. Houston Merrett, Paul A. O'Leary, Walter L. Palmer, Hobart A. Reimann, Cyrus C. Sturgis, Robert H. Williams. Pp. 672. Price \$10. Philadelphia and London: W. B. Saunders Company, 1949.

**ATLAS OF ORAL AND FACIAL LESIONS AND COLOR FILM LIBRARY.** By Ralph Howard Brodsky, D.M.D., consulting oral surgeon of the Department of Hospitals, New York, lecturer in stomatology, Graduate School of Medicine, New York University, associate dentist to the Mt. Sinai Hospital, New York. Pp. 129, with 100 color slides. Price \$80. Baltimore: Williams & Wilkins Company, 1949.

This is a teaching unit consisting of a cloth-bound text of 127 pages and a case with 100 color slides bearing numbers that correspond to numbers in the text. In the book is a description of each slide with diagrams indicating the particular condition each slide illustrates. The book and the slides should be of value in teaching and in diagnosis.

**AN ATLAS OF BONE-MARROW PATHOLOGY.** By M. C. G. Israels, M.Sc., M.D., M.R.C.P., lecturer and deputy director of the department of haematology of the University and Royal Infirmary, Manchester. Illustrations by D. Davison, medical artist to the University of Manchester. Price \$6.50. Pp. 79, with 3 figures and 12 color plates. New York: Grune & Stratton, 1948.

This little volume is designed to induce pathologists to examine the bone marrow and to tell the clinician the results of such examinations. The book is in two sections. The first deals with technique and the identification of the various cell types. The second part describes the marrow patterns found in different diseases. The clinician will welcome the comprehensive tabulation of the typical findings in the various blood disorders. The pathologist may consult the table which lists the diagnoses compatible with different marrow pictures. The first seven color plates depict the normal and the pathologic cells. The last five plates contain twenty characteristic marrow patterns. The illustrations are excellent. This atlas can be highly recommended to any student of blood diseases.

OBSERVATIONS ON THE PATHOLOGY OF HYDROCEPHALUS By Dorothy S Russell  
Medical Research Council Special Report Series no 265 Price 6 shillings Pp 138,  
with 90 illustrations London His Majesty's Stationery Office, 1949

This report on hydrocephalus is a thorough and lucid discourse on the subject. Interest and information are added by critical reviews of other publications. The author in meeting Spiller's reproach "that actually observed lesions (of hydrocephalus) are much rarer than theories explanatory of the causes of hydrocephalus" presents a wealth of material which refutes the "ideopathic" origin of hydrocephalus. She systematically classifies her cases according to etiology. Maldevelopments are critically analyzed, and reasons for disagreeing with current theories are objectively presented. Inflammation is fully considered, the author commenting on the high incidence of *Bacillus coli meningitis* in the newborn. The entire problem of neonatal meningitis is considered at great length. Syphilis as a cause of hydrocephalus is rare in the author's experience. Numerous case histories, complete with postmortem findings and excellent illustrations, and an extensive bibliography are presented. Pathologists and neurologists will find it a useful text and reference source.

DIE PATHOLOGISCH-ANATOMISCHEN GRUNDLAGEN DER ALLERGIE Von Doz  
Dr med Wilhelm Eickhoff, Rheine, Westphalia Pp 95, with 40 illustrations  
Stuttgart Georg Thieme Verlag, 1948

The purpose of this booklet is to introduce the beginner into the complex field of allergy by a presentation of the pathologic-anatomic features of the subject. The book is divided into two parts: experimental allergy and parallergy in the animal, and a short review of knowledge of human allergy as applied to immunization phenomena. There are many good photographs of the gross and histologic aspects of allergic phenomena. Unfortunately, no references to the literature are given. It is a well organized introduction to a difficult subject from a new point of view and should be of value to everybody interested in the theoretic basis of allergy.

EVALUATION OF CHEMOTHERAPEUTIC AGENTS Edited by Colin M MacLeod  
Symposium held at the New York Academy of Medicine, March 25 and 26, 1948  
Price \$4 Pp 205 New York Columbia University Press, 1949

The book contains the fourteen papers of the symposium. The authors are active investigators in chemotherapy. The papers deal instructively with general factors concerned in chemotherapy rather than with specific compounds for particular diseases.

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